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Relative toxicity of several non-protein nitrogen feeding compounds for lambs

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RELATIVE TOXICITY OF SEVERAL NON-PROTEIN NITROGEN FEEDING COMPOUNDS FOR LAMBS

by

Ward William Repp

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In Charge of Major Work

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Head of Major Department

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TABLE OF CONTENTS

Page

INTRODUCTION

One of the most important problems in the field of ruminant nutrition when considered both from the academic and practical standpoints is the problem of determining the process by which ruminant animals are able to utilize nitrogenous materials. Nitrogen, occurring as it does in all proteins, must be considered a vital nutrient essential to all living processes.

In view of the competition which exists between ruminant and non-ruminant animals for concentrate feeds it appears that the over-all agricultural economy would be served if ruminants could be fed rations which contain increasing proportions of roughage and correspondingly lower proportions of concentrate feeds. A vast potential of low quality roughages such as corn cobs, cornstalks and poor quality hay, pasture and range grasses exists which may be used by ruminant animals in the production of relatively low-priced beef and mutton. In order for cattle and sheep to utilize roughages (and especially low quality roughages) efficiently, however, several known and probably many unidentified nutrients and growth factors must be included in the ration. In addition to supplementation with growth factors, minerals and hormones, rations containing low quality roughages must be supplemented with a nitrogen

source. Ruminants are physiologically adapted to the utilization of relatively simple nitrogen compounds since ingested feed must first traverse the rumen where extensive bacterial fermentation takes place. These simple nitrogenous compounds are utilized in the synthesis of bacterial protein which in turn undergoes digestion and absorption further down the digestive tract of the animal.

In trying to find a suitable non-protein nitrogen feeding compound for ruminants, several considerations are important. It is important that such a compound be non-toxic. It would also be desirable that the non-nitrogen portion of the compound be utilizable by the animal. From an economic standpoint the cost of producing an ideal non-protein nitrogen compound should be low.

The non-protein nitrogen compound in most common use today is urea. This study was conducted to find a compound which was less toxic than urea and one which might contain a non-nitrogen portion which would be utilizable in the animal body. Subordinate purposes of the study included finding ways of alleviating symptoms of toxicity caused by administration of non-protein nitrogen compounds and finding the capabilities of rumen microorganisms in adapting themselves to utilization of these compounds.

REVIEW OF LITERATURE

Use of Non-protein Nitrogen Compounds Other than Urea in Ruminant Nutrition

Since the problem of the utilization of urea as a protein substitute in ruminant rations has been adequately reviewed by Krebs (22), Terroine (37) and Reid (33), no attempt will be made to review the literature on this subject except to state that these authors conclude that urea may be utilized successfully by ruminants as at least a partial protein replacement for growth, fattening and lactation.

Non-protein nitrogen (NPN) compounds other than urea have been used as nitrogen sources in the nutrition of ruminants. Many of the early experiments with these compounds were conducted in Europe where the cost of protein concentrates is high.

Ammonium bicarbonate is one of the NPN compounds with which considerable experimental work has been done. Ehrenberg *et al.* (11) reported that for short periods protein could be very largely and in some instances completely replaced by ammonium bicarbonate in the feeding of milk cows. In a similar experiment Ehrenberg and Briese (12) found that an experimental group of milk cows receiving ammonium

bicarbonate as a substitute for protein gained slightly more in body weight and produced essentially as much milk as the control group receiving conventional protein. The addition of ammonium bicarbonate to silage, Ehrenberg (13), resulted in the formation of ammonium salts of the organic acids and enrichment of the silage nitrogen, although cows fed on the enriched silage gave smaller amounts of milk with lower fat content than those receiving an equivalent amount of nitrogen in the form of high protein feed. Using milking goats as experimental animals, Ziemer (44) determined that ammonium bicarbonate could replace about 50 per cent of the protein in a ration designed to maintain milk production.

Other German workers, Windheuser et al. (41), studied the influence of admixtures of ammonium bicarbonate and ammonium carbonate upon the quality of grass and maize silage. The digestion coefficients of pure protein were higher and the losses of pure protein and digestible protein were lower, but the loss of dry matter was somewhat higher than in the corresponding silages without admixture.

Experiments were performed by Kirsch and Jantzon (21) on three milk cows over a period of 30 days on a ration of clover silage, molassed beet slices, potato flakes and ammonium bicarbonate equivalent to about 25 per cent of the total nitrogen of the ration. The total nitrogen was

adjusted so that both the amides of the silage and the ammonium bicarbonate were required to meet the nitrogen requirements. It was concluded that the nitrogen of bicarbonate was utilized since body weight and milk yield showed only insignificant changes. In a metabolism experiment on a wether, utilization of the non-protein nitrogen was also indicated.

In this country the first work with ammonium bicarbonate was done by Hart and co-workers (17) at the University of Wisconsin. In two separate experiments with ten growing calves in which 43 per cent of the total nitrogen of the ration was furnished by ammonium bicarbonate, utilization of the non-protein nitrogen was indicated by growth nearly comparable to the control calves over a 16-week period.

Ammonium acetate has also been used to replace protein in ruminant rations. In a short-time experiment of three weeks duration (30) up to 50 per cent of the fodder protein was replaced with ammonium acetate without detriment to the milk yield of goats. Working with male goats, Kametaka et al. (20) reported that positive nitrogen balance was maintained and that percentages of digestibility of crude protein, true protein, organic matter and fat were comparable to the basal ration, with crude fiber digestibility somewhat lower when ammonium nitrate was used as a protein substitute.

The experimental results with the use of glycine as a protein substitute have been variable. In a trial with milk cows lasting 65 days, Schmidt et al. (36) found that the effect of replacing oil cake with glycine was to decrease milk yield and body weight to nearly the same extent as did the low-nitrogen control ration. Although glycine did not spare protein in this study it did appreciably increase the fat content of the milk. In a similar experiment with milk cows in which the oil cake of the ration was replaced for three months with glycine, Ehrenberg et al. (14) found that in terms of milk production, glycine had about 30 per cent of the efficiency of oil cake.

In unpublished research Repp (34) found that in feeding ammonium succinate and guanidine carbonate in increasing amounts during ten six-day periods such that in the final period 70 per cent of the total nitrogen was furnished by these compounds, lambs gained in body weight throughout the trial. Lambs fed ammonium sulfate and ammonium sulfamate on the same schedule as above were unable to maintain body weight.

In addition to feeding trials, several in vitro studies have been made to evaluate non-protein nitrogen compounds as nitrogen sources for rumen bacteria. One of the most extensive of these studies was made by Belasco (1). A vast number of non-protein nitrogen compounds were tested using

cellulose digestion, nitrogen utilization and bacterial growth as criteria of effectiveness as nitrogen sources. The nitrogen from numerous organic and inorganic non-protein nitrogen compounds was found to be highly available. The ammonium salts of formic, alpha-ketoglutaric, malic, succinic and lactic acids showed higher rates of nitrogen utilization than urea and other ammonium salts possibly because of the stimulatory effect of the organic fragments of these salts upon nitrogen fixation by the bacteria. High levels of cellulose digestion were obtained when the carbonate, hydrochloride and acetate salts of guanidine were used as the sole nitrogen source in the artificial rumen. Creatine and creatinine, examples of purines, also proved to be effective sources of nitrogen. Good bacterial growth occurred when nitrogen was supplied as the amides of formic, acetic, propionic and n-butyric acids. Interestingly the amide of glycine proved to be an effective nitrogen source whereas glycine itself did not provide nitrogen to the bacteria. The diamides of dicarboxylic acids including oxamide, glutaramide, malonamide, diglycolamide and adipamide did not provide available nitrogen. Such purines as uric acid and allantoin were found to supply nitrogen to the rumen bacteria.

Loosli *et al.* (25) after placing sheep on a purified diet containing glycine as the only source of nitrogen,

analyzed rumen material for the presence of the ten essential amino acids. This experiment indicated synthesis of all ten essential amino acids by the rumen bacteria although at a lower rate than when urea was used as the sole nitrogen source. Pearson and Smith (32) reported that when rumen bacteria from a fistulated steer were incubated with ammonium bicarbonate, ammonium sulfate and urea, the amount of protein synthesis as measured by precipitable protein was nearly twice as great with urea and ammonium bicarbonate as with ammonium sulfate. In a similar type experiment Hudman et al. (19) reported that ammonium acetate was as effective as urea in stimulating protein synthesis, while less synthesis was noted when ammonium citrate and ammonium bicarbonate supplied the nitrogen. The use of hydrochloric acid to maintain the pH of the flasks containing ammonium bicarbonate may have acted to lessen the utilization of this compound in protein synthesis.

Toxicity of Urea and Other Non-protein Nitrogen Compounds

The animals employed by Hart (17) were slaughtered after having received rations containing urea, ammonium bicarbonate and casein for 12 months. The animal which received 4.3 per cent of urea manifested diuresis during the

feeding trials and upon necropsy was found to have hypertrophied kidneys and necrotic areas in the liver. Evidence of slight kidney damage was observed in the animal whose ration contained 2.8 per cent of urea. No kidney damage was noted among the animals receiving rations containing 1.4 per cent urea, 11.1 per cent ammonium bicarbonate or 11 per cent casein. Work et al. (43) detected no damage to kidneys or livers of fattening steers fed as much as 2.29 per cent of urea.

The work of the Wisconsin (17) and Hawaiian groups (43) was significant since it appeared to demonstrate that a level of urea in the ration between 2.29 per cent and 2.8 per cent is harmful to cattle. Harris and Mitchell (16) reported that feeding wethers rations containing as much as 3.16 per cent urea did not exert any toxic effect over a feeding period of 110 days and that there was no histological evidence of kidney damage.

Recently, the effects of orally administered urea to cattle and sheep have been studied by Dinning et al. (10). These workers observed that oral administration of 40 grams of urea in water solution to a sheep produced a rapid rise in ammonia and urea of the portal blood. The ammonia level in the portal blood continued to increase during a two-hour observation period and reached a maximum of 8.4 milligrams per cent indicating hydrolysis of urea in the rumen and

absorption of ammonia through the rumen wall. Twenty minutes after drenching cattle with 116 grams or more of urea, symptoms of toxicity were observed including ataxia, severe tetany, a retarded respiratory rate and salivation. Ataxia appeared in the steers when the ammonia level of the systemic blood reached 2.5 milligrams per cent. Symptoms of toxicity became more severe as the concentration of ammonia increased and death occurred when the ammonia level reached about 4 milligrams per cent. When mixed in the feed amounts of urea up to 400 grams daily were consumed by steers without ill effects.

The most extensive study made of acute urea toxicosis in sheep was reported by Clark et al. (8). The clinical symptoms occurred 30 to 60 minutes after administering as little as 10 grams of urea directly into the rumen. The symptoms of toxicity were described by these workers as: dullness followed by hyperaesthesia, severe twitching of the musculature of the whole body, and bloat, followed by tetany of the skeletal musculature prostration of the animal with legs extended, labored breathing and regurgitation of ruminal contents just prior to death. Death was found to be caused by an acute circulatory failure with generalized venous stasis. Hemorrhage under the epi- and endocardium were almost always found. Marked fatty degeneration of the liver and kidney was observed in all animals necropsied.

The cause of death was not certainly ascertained. However, intravenous injections of urea or ammonium hydroxide did not produce symptoms of toxicity. Dilute acetic acid administered either intravenously or directly into the rumen prevented fatal toxicity even when symptoms were advanced. Sheep consuming alfalfa hay tolerated larger quantities of urea than those receiving poor-quality grass hay. An interesting observation was made by Weinstein and McDonald (39) when they found that urea in concentrations of from 3 to 12 per cent of the medium had a bacteriostatic effect upon certain types of bacteria. It would seem that this aspect of urea toxicity merits further investigation.

No ill effects resulted from feeding five steer calves 0.02 to 0.06 pound urea per 100 pounds of body weight daily in a preliminary study conducted by Briggs et al. (2). Gross and histological examinations of five additional steers fed 0.4 pound urea daily for 14 days showed no evidence of kidney damage. From results of additional experiments Briggs et al. (3) concluded that yearling steers refused to eat enough feed containing 8 per cent urea to cause kidney damage.

The feeding of 0.6 pound urea daily to dairy calves weighing 300 to 500 pounds (27) caused no adverse effect other than diuresis. Corn silage made with the addition of 10 pounds of urea per ton of corn ensiled was fed along

with concentrates and hay to dairy cows by Woodward et al. (42). One Holstein cow ate an average of 104 pounds of silage containing 1.17 pounds of urea daily. Postmortem examination revealed no evidence of harm to these cows. No difference in breeding history, composition or flavor of milk or composition of milk was found among cows fed a concentrate mix containing 3 per cent urea as compared to the basal ration containing linseed meal (35). In another feeding trial with dairy cattle (29) in which 25 per cent of the total nitrogen was furnished by urea, the urea content of the milk closely approximated that of the blood and never exceeded 28 milligrams per 100 milliliters, an amount which would not have any deleterious effect upon the consumer.

Holstein calves were grown for 222 days on a ration containing ammoniated sugar beet pulp in which approximately 45 per cent of the total nitrogen of the ration was supplied by liquid ammonia incorporated into the beet pulp (26). No diuresis occurred in these calves and analysis of blood for 15 different constituents including urea and ammonia at three times during the trial revealed no abnormalities. The internal organs passed federal inspection at slaughter. Rib, liver and kidney samples were found to be normal in protein, and the color and flavor of the meat were also normal.

Adaptability of Rumen Microorganisms to Added Nutrients or Substances

Of the areas of study included in this thesis problem probably none has been less completely investigated than that of the adaptability of rumen microorganisms to added nutrients or substances. Several studies have been made which are closely associated with the problem. Gall et al. (15) reported that a calf fed a purified diet containing urea showed a rumen flora which varied microscopically from that of animals fed hay and grain. It was reported by Williams et al. (40) that ruminal bacterial numbers were significantly lower from lambs fed a urea diet than from lambs fed rations containing egg protein, casein or urea plus methionine.

Chance et al. (6) studied the effect of aureomycin on the total bacterial counts and on the streptococci and coliform groups from the rumen contents of two fistulated steers. It was found that a definite increase occurred in the total bacterial count of the rumen contents when aureomycin was included in the ration. The number of rumen streptococci decreased, whereas the number of coliform organisms remained approximately the same in one steer and increased in the other. Bacteriological studies of fresh rumen material from heifers fed aureomycin for 30 days (28)

revealed that total counts were about the same in all lots, but that the types of organisms found in the heifers receiving aureomycin were much less diverse, suggesting that the normal bacterial flora had been disturbed.

Whereas the experiments reported above do indicate that adaptations in types and numbers of bacteria do occur when changes are made in the diet of ruminants, they do not offer evidence of specific adaptation to utilization of or resistance to the added nutrient or substance. Lodge (24) has demonstrated specific adaptation of rumen microorganisms to the addition of aureomycin. In in vitro studies he reported that the addition of 2.4, 1.6, 0.8 and 0.4 micrograms of aureomycin per milliliter of fermentation mixture in the artificial rumen severely inhibited cellulose digestion by microorganisms from cows not previously fed aureomycin. The same additions had comparatively little effect on the digestion of cellulose by the inoculum from the aureomycin-fed cows.

EXPERIMENTAL

Influence of Oral Administration of Non-protein Nitrogen Feeding Compounds upon Blood Ammonia and Urea Levels in Lambs

The amount of urea recommended for general use in ruminant rations is limited to low levels principally because of the possibility of toxicity developing when high levels are fed. This toxicity is believed to be due to rapid liberation in the rumen of the ammonia from the urea with subsequent absorption of ammonia into the portal blood resulting in an ammonia toxicity. Dinning et al. (10) have shown an increase in the portal and systemic blood ammonia following administration of a large single dose of urea to one lamb. Rises in the urea and ammonia of the systemic blood were also noted when steers were given a single dose of 100 grams or more of urea. Clark et al. (8) in studying acute urea toxicity in lambs suggested that the cause of death was not due to absorbed ammonia but possibly to the production of some toxic product of rumen fermentation.

The purpose of this part of the study was to gain more information regarding the mechanism of urea toxicity and amounts required to produce toxicity. A second purpose was to determine whether other non-protein nitrogen compounds

might be less toxic than urea and in this respect superior to urea as nitrogen sources in ruminant rations. The compounds tested were urea, ammonium acetate, ammonium formate, ammonium propionate and propionamide with limited observations being made on ammonium succinate, formamide, guanidine carbonate, biuret, casein and glycine.

Materials and methods

For the initial studies on the first five compounds listed above, 30 wether lambs of fine wool breeding, weighing about 75 pounds each were used. Limited observations were also made with the remaining compounds using 15 crossbred lambs. All lambs were fed a ration consisting of .25 pound soybean oil meal, .75 pound cracked shelled corn and all the red clover hay of good quality they would consume. In testing the first five compounds the 30 wether lambs were allotted at random into groups of six lambs each. The 15 crossbred lambs were also allotted to treatment at random.

All compounds tested were administered on an equivalent nitrogen basis, with urea being used as a standard. The compounds were put into solution in about a pint of water and given by stomach tube. Precaution was taken to be sure that the end of the stomach tube was inserted into the rumen. For the initial studies feed was withheld for

18 hours prior to drenching, but water was available at all times. The initial dose level for testing was 15 grams of urea (or its nitrogen equivalent of the other compounds tested) per 100 pounds of body weight. Clark et al. (8) have shown that as little as 18 grams of urea in a single dose per 100 pounds body weight will cause death in lambs. Additional dose levels beyond the first dose were increased by increments of 5 grams of urea equivalent until at least one-half of the six lambs allotted per compound succumbed to treatment. This was then accepted as the toxic level for the compound. Due to the limited number of animals available it became necessary to drench some individual animals more than one time. In these cases at least ten days were allowed between drenches.

Preliminary studies were carried out in order to determine the time after administration of the non-protein nitrogen compound that the blood ammonia reached its peak. Accordingly, four lambs were drenched with 20 grams urea equivalent of urea, ammonium formate, ammonium propionate and ammonium acetate. The lambs were bled just prior to drenching and at intervals of 10, 20, 30, 50, 70 and 180 minutes after drenching. Blood samples were taken from the jugular vein. As indicated in Figure 1 and Table 1 it was found that blood ammonia values were at a maximum at about

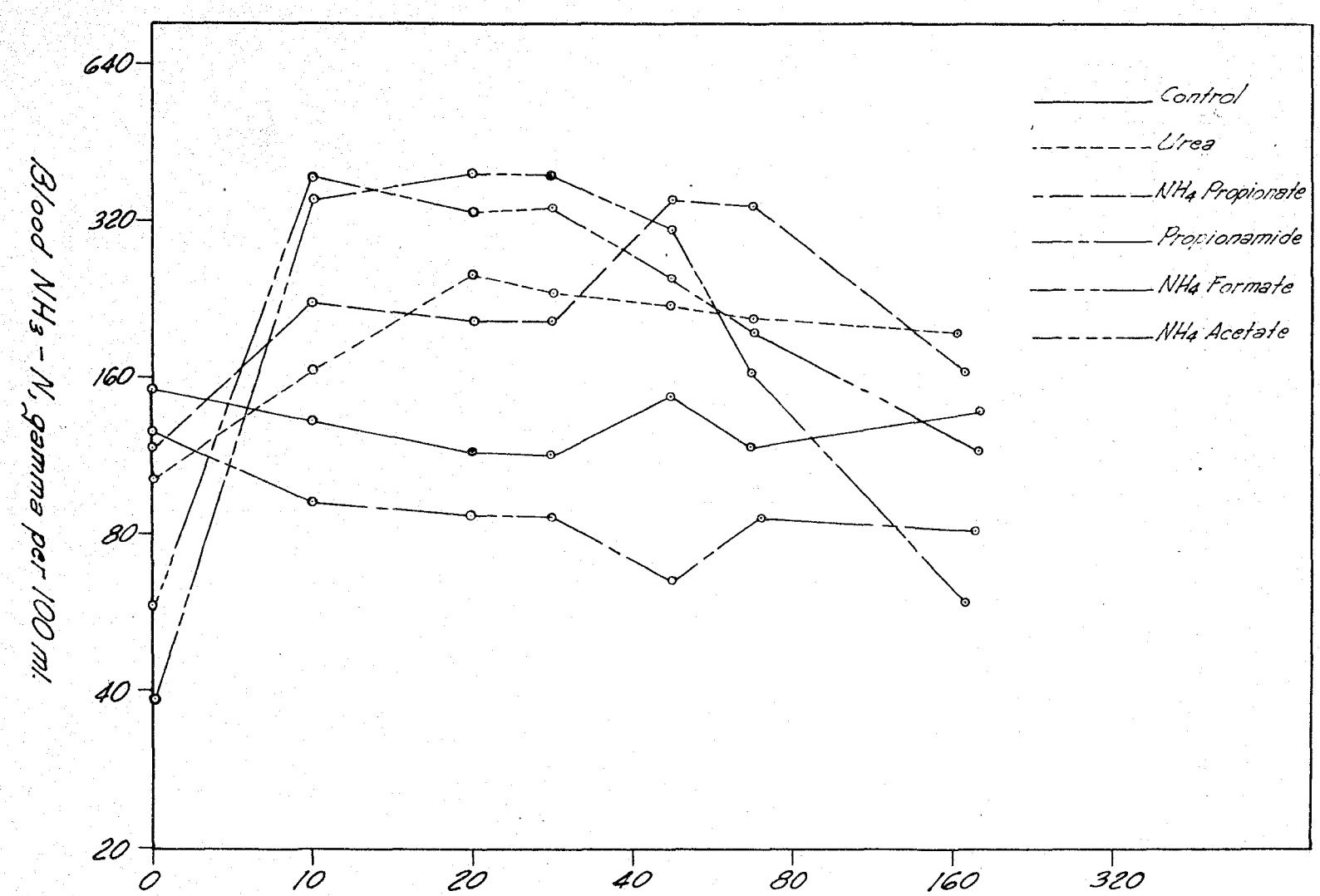


Figure 1. Blood NH_3 - N Levels Following Sub-lethal Doses of NPN Compounds to Lambs

Table 1. Blood NH_3-N levels following sub-lethal doses of NPN compounds administered orally to lambs (micrograms per 100 milliliters)

Bleeding intervals	Compounds administered					
	Urea	Ammonium propionate	Propionamide	Ammonium formate	Ammonium acetate	Control
0 time	110	123	129	39	62	157
+10 mins.	162	238	97	361	410	139
+20 mins.	264	217	90	409	347	121
+30 mins.	245	217	91	406	350	120
+50 mins.	231	364	68	311	262	149
+70 mins.	217	334	86	161	203	123
+180 mins.	204	163	80	64	119	141

30 minutes after drenching. As the result all lambs drenched with test compounds were bled just prior to drenching and 30 minutes after drenching unless clinical symptoms of toxicity occurred in which case samples were taken more often. While it is realized that analysis of the systemic blood probably does not accurately measure the ammonia being absorbed through the rumen wall it does appear to give a good estimate which can be employed to advantage for routine determinations.

In Figure 2 and Table 2 are recorded the effects of the 20 gram urea equivalent doses upon blood urea values. It will be noted that the rate of increase in blood urea was much slower than that of blood ammonia and that the blood urea values continued to rise throughout the three-hour period during which samples were taken. Blood ammonia was determined by a micro-diffusion method, Conway (9), and urea by an aeration method, Van Slyke and Cullen (38).

Based upon the findings with the first five compounds, limited observations were made on the last six compounds in regard to their effect upon blood ammonia and urea. Limited data were also collected on therapeutic measures attempted in an effort to prevent or alleviate symptoms of toxicity. Several animals were also drenched with urea after being withheld from feed for varying lengths of time. Blood ammonia and urea levels were thus determined in checking the effect of starvation. Blood ammonia and urea levels

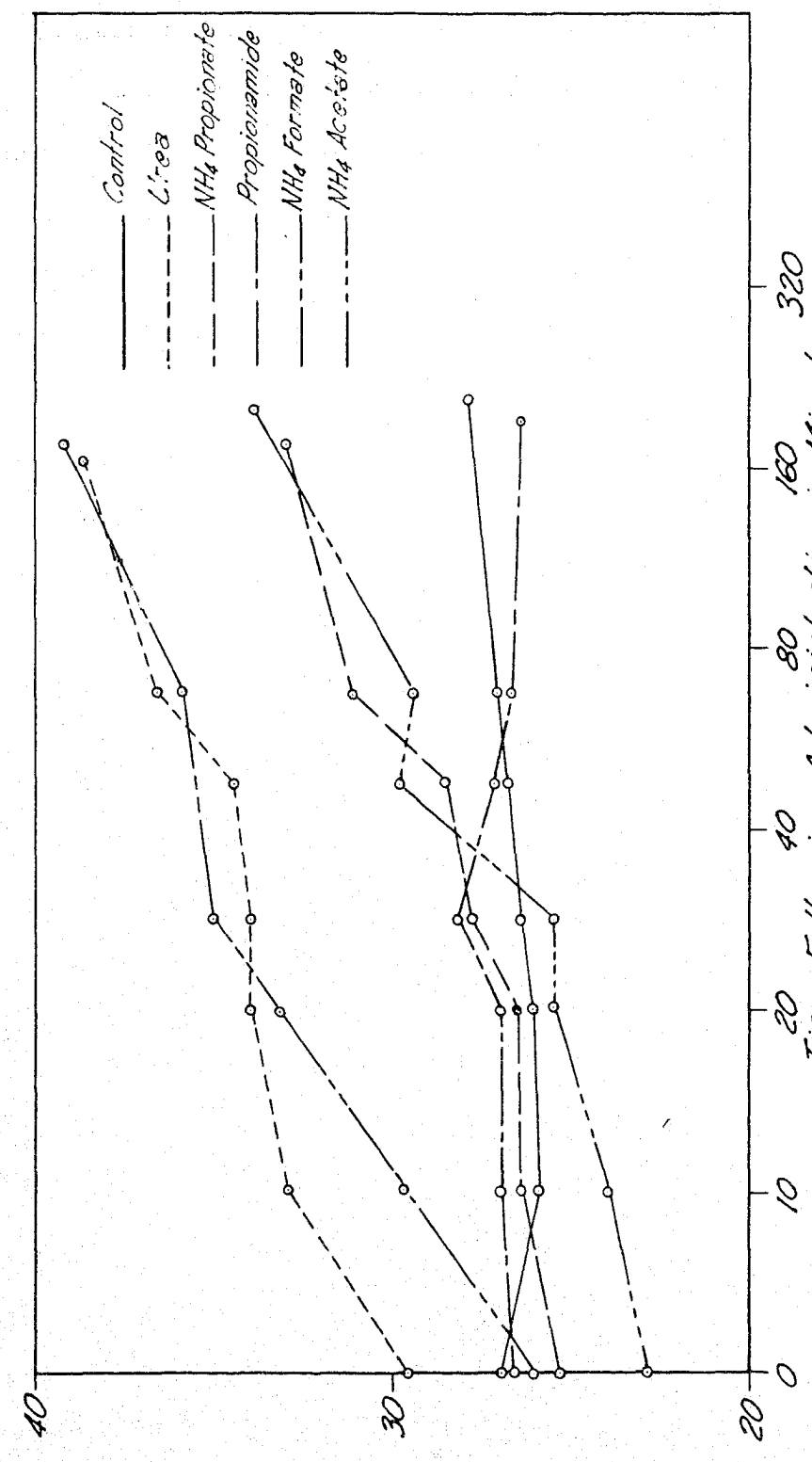


Figure 2. Blood Urea-N Levels Following Sub-lethal Doses of NPN Compounds to Lambs

Table 2. Blood urea-N levels following sub-lethal doses of NPN compounds administered orally to lambs (milligrams per 100 milliliters)

Bleeding intervals	Compounds administered					
	Urea	Ammonium propionate	Propionamide	Ammonium formate	Ammonium acetate	Control
0 time	29.61	25.26	26.56	26.52	22.84	26.46
+10 mins.	32.84	26.27	26.97		23.94	25.88
+20 mins.	33.86	26.50	26.86		25.50	26.06
+30 mins.	33.90	27.74	28.06	34.10	25.52	26.31
+50 mins.	34.56	28.46	27.12		29.82	26.73
+70 mins.	36.06	31.18	26.70	36.06	29.29	26.86
+180 mins.	38.82	32.81	26.42	39.43	33.78	27.84

were also checked on four animals administered propionamide as a drench after having received this compound in their ration for 49 days. All animals that died as a result of the tests were necropsied. All values given for blood ammonia and blood urea are micrograms of NH_3-nitrogen (NH_3-N) and milligrams of urea-nitrogen (urea-N) respectively per 100 milliliters of whole blood.

Results and discussion

In Table 3 are recorded the maximum blood NH_3-N values obtained at the various levels of administration of urea, ammonium propionate, ammonium acetate and ammonium formate. The blood NH_3-N levels at which clinical symptoms of toxicity or death resulted are also indicated in this table. It will be noted that no clinical symptoms of toxicity occurred until the blood NH_3-N level rose to approximately 1000 micrograms per 100 milliliters. Between this level and 1158 micrograms per 100 milliliters the animals exhibited ataxia but recovered with one exception. This lamb received ammonium propionate and the maximum recorded blood NH_3-N value was 1076 micrograms per 100 milliliters; however, he showed severe symptoms of toxicity for a period much longer than normally observed and finally died nearly six hours after drenching. All other lambs whose blood NH_3-H levels rose

Table 3. Maximum recorded blood NH_3-N values following oral administration of NPN compounds to lambs at the indicated doses of urea equivalent per 100 pounds body weight (gamma per 100 milliliters)

	0 gms.[a]	15 gms.[b]	20 gms.	25 gms.	30 gms.	35 gms.	40 gms.
Urea	147	146 268	139 155 264	526 425	663 616	441 1158*	595 2893** 810 3585**
Ammonium propionate	148	329 288	179 172 364	2519** 458	1012* 2925** 473	717 1076** 670	684
Ammonium formate	138	175 185	279 272 409	704 588	874 413	758 1003	3355** 2656** 806 3304**
Ammonium acetate	132	175 159	275 280 410	525 692	1146* 389	889 479	3114** 2045** 2872**

*Animals exhibited ataxia but recovered.
**Animals died.

[a]The values for 0 grams are the blood NH_3-N levels prior to drenching and for each compound is an average of the same number of observations given for all test levels of that compound.
[b]Each value given for test levels represents an observation on an individual animal.

higher than 1158 micrograms per 100 milliliters developed symptoms of acute toxicity and subsequently died.

Fatal toxicity was first encountered with a lamb drenched with ammonium propionate at the 25 gram urea equivalent level. One lamb which received this compound at the 30 gram and a lamb receiving it at the 35 gram level also died, although an additional lamb receiving ammonium propionate at the 40 gram level did not show any clinical signs of toxicity. In the case of urea, ammonium formate and ammonium acetate, fatal toxicity was encountered at the 40 gram urea equivalent level. The number of animals succumbing to treatment at this level is shown in Table 3. With all the lambs that died due to treatment with these three compounds blood NH_3-N levels rose to at least 2045 micrograms per 100 milliliters shortly before death.

Figure 3 illustrates the regression of maximum blood NH_3-N values upon dose levels of the toxic compounds. This regression represents 118 individual observations. The values used in this regression are the highest blood NH_3-N values observed at any time following drenching of a lamb with a test compound. The rate of increase in blood NH_3-N values following the administration of toxic doses is presented in Figure 4 and Table 4. These summaries show that when lethal doses were administered the blood NH_3-N values

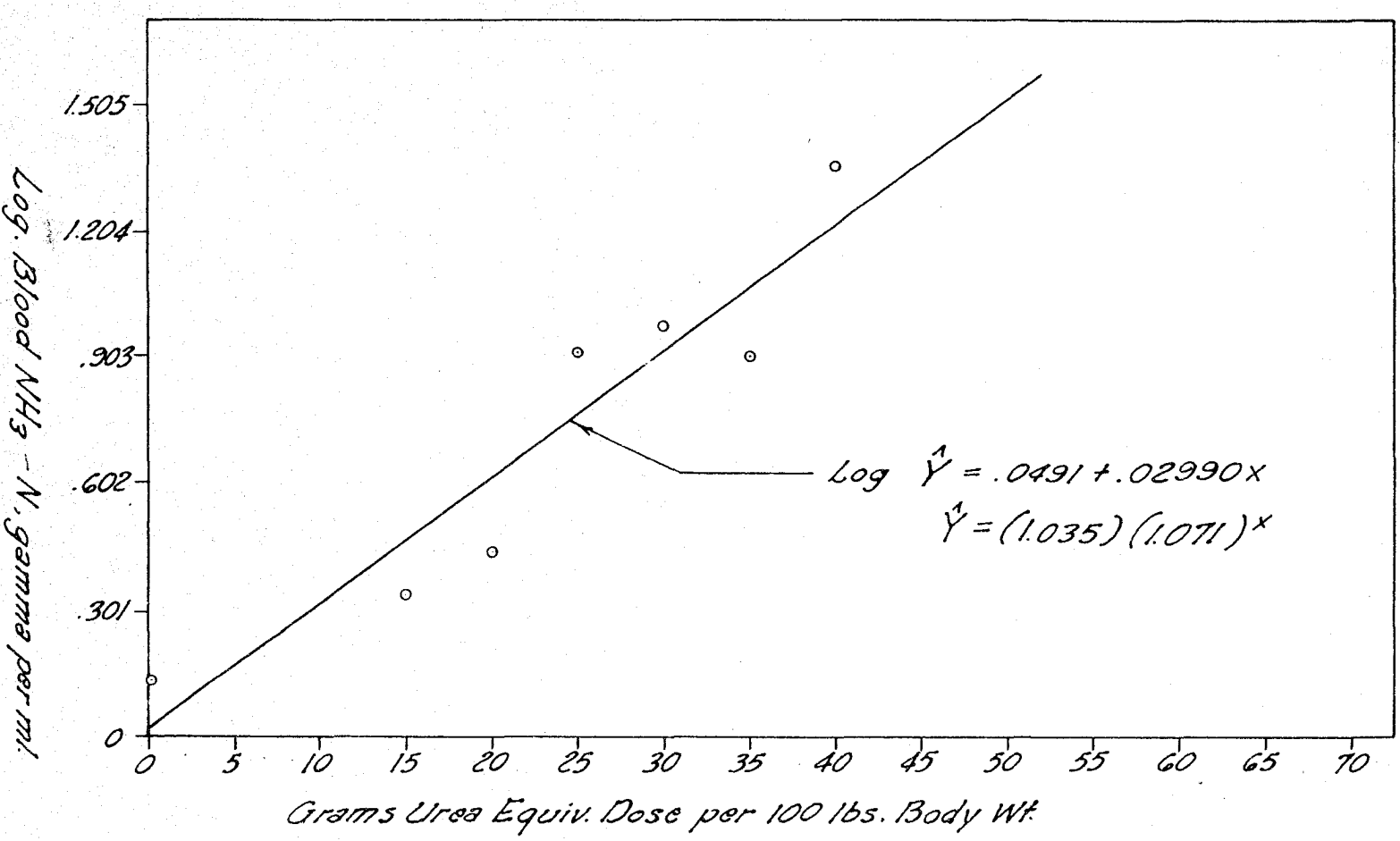


Figure 3. Regression of Maximum Blood NH_3-N Values on Dose Levels of Toxic Compounds' to Lambs'

'Compounds are Urea, Ammonium Formate, Ammonium Acetate and Ammonium Propionate

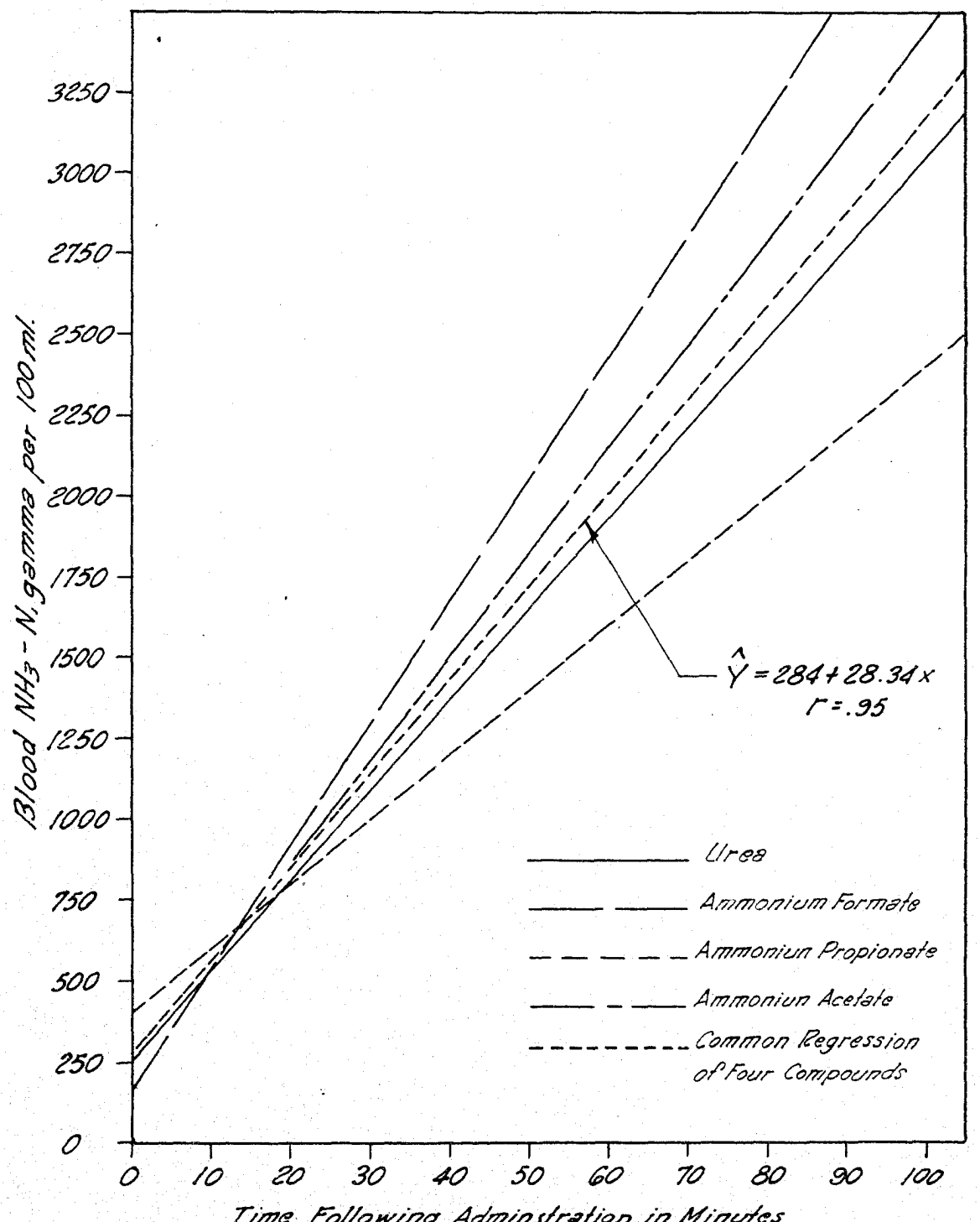


Figure 4. Regressions of Blood NH_3 - N Levels on Time Following Administration of Toxic Compounds to Lambs

Table 4. Blood NH_3-N following oral administration of lethal doses of NPN compounds to lambs (gamma per 100 milliliters)

Compounds administered	Bleeding intervals					
	0 time	+30 mins.	+60 mins.	+72 mins.	+80 mins.	+90 mins.
Urea	187	1840	2862			3585
	181	896	1574			2893
	189	902	1274			1739
Ammonium formate	140	1478	2870		3304	
	143	1619	2573		3355	
	151	948	1251		2656	
Ammonium propionate	183	1645	1981			2519
	147	1338	1754			2925
	146	915	992			1047
Ammonium acetate	147	1200	1451	2872		
	153	1520	2346	3114		
	167	1491	1767	2045		

rose rapidly. The increase in blood NH_3-N continued until the animals died. When toxic levels of the compounds were administered symptoms of toxicity appeared 30 to 45 minutes after drenching, with death usually occurring 90 to 120 minutes after drenching.

The rise in blood urea-N following administration of lethal doses of the four toxic compounds proceeded at a slower rate than did the ammonia values. This is shown in Figure 5 and Table 5.

Data collected showed that the blood ammonia values of animals which survived treatment with high levels of the toxic compounds started decreasing about 60 minutes after drenching; however, blood urea values were still increasing 80 minutes after drenching. Observations on animals surviving after having received toxic and nearly fatal doses of urea, ammonium propionate, ammonium acetate and ammonium formate showed that at 24 hours the blood urea values had not returned to normal (Figure 6 and Table 6).

Extensive observations were also made on propionamide as it was included with the first five compounds tested. However, this compound proved to be non-toxic. Fourteen observations were made with this compound at levels from 15 to 80 grams urea equivalent. The effect of this compound on blood ammonia and urea is shown in Figure 7 and Tables 7a and 7b.

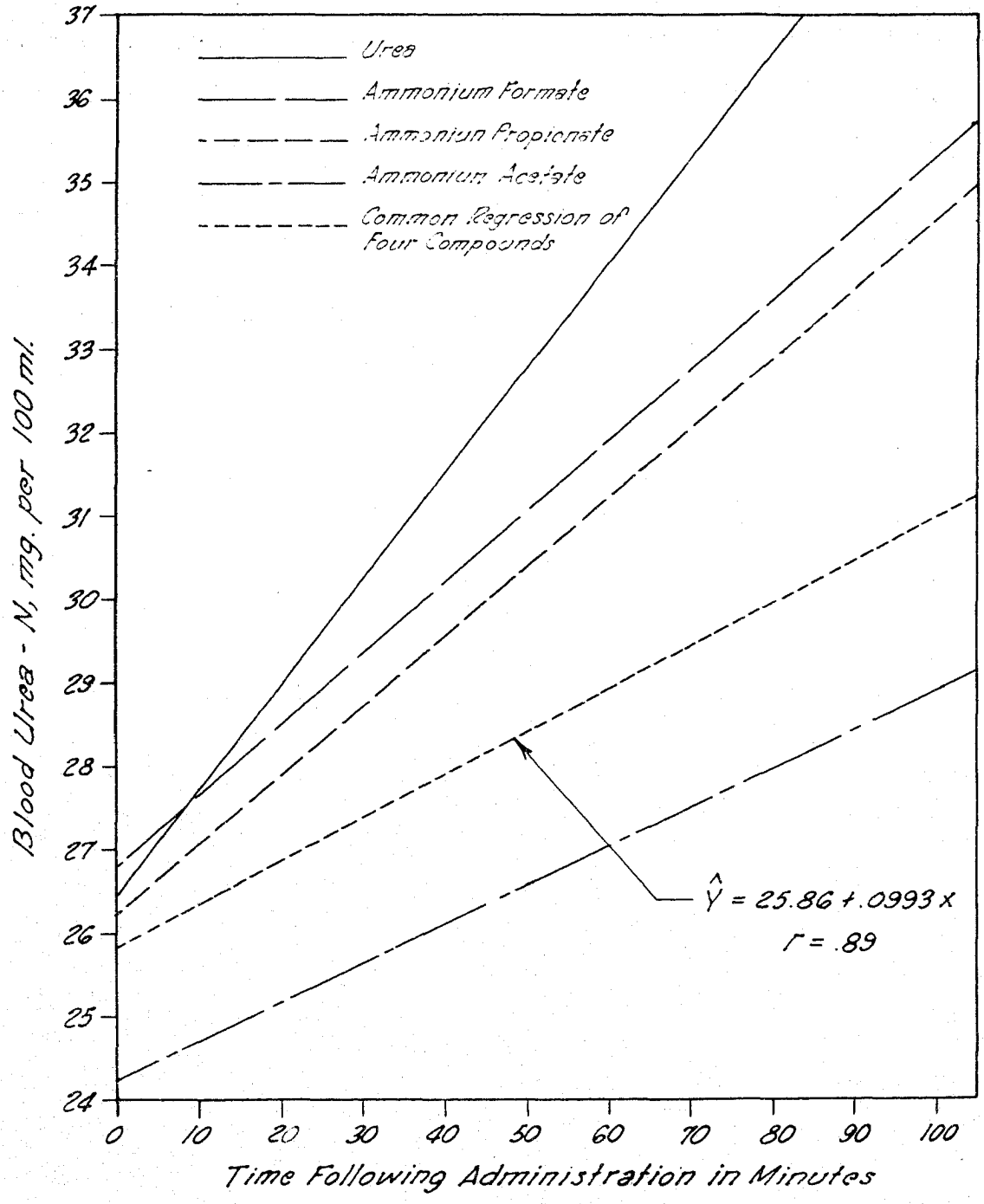


Figure 5. Regressions of Blood Urea - N on Time Following Administration of Lethal Doses of Toxic Compounds to Lambs

Table 5. Blood urea-N following oral administration of lethal doses of NPN compounds to lambs (milligrams per 100 milliliters)

Compounds administered	Bleeding intervals					
	0 time	+30 mins.	+60 mins.	+72 mins.	+80 mins.	+90 mins.
Urea	22.60	27.68	30.26			34.73
	30.48	38.58	41.74			43.39
	22.74	29.11	31.60			32.36
Ammonium formate	29.50	33.55	35.28		37.57	
	23.00	25.30	27.92		28.03	
	27.14	29.97	33.58		33.96	
Ammonium propionate	27.48	32.00	49.29			37.54
	25.40	28.40	29.64			29.96
	24.60	25.09	26.94			30.20
Ammonium acetate	18.60	20.78	23.82	25.43		
	26.04	32.78	34.66	35.26		
	26.80	30.97	28.79	32.69		

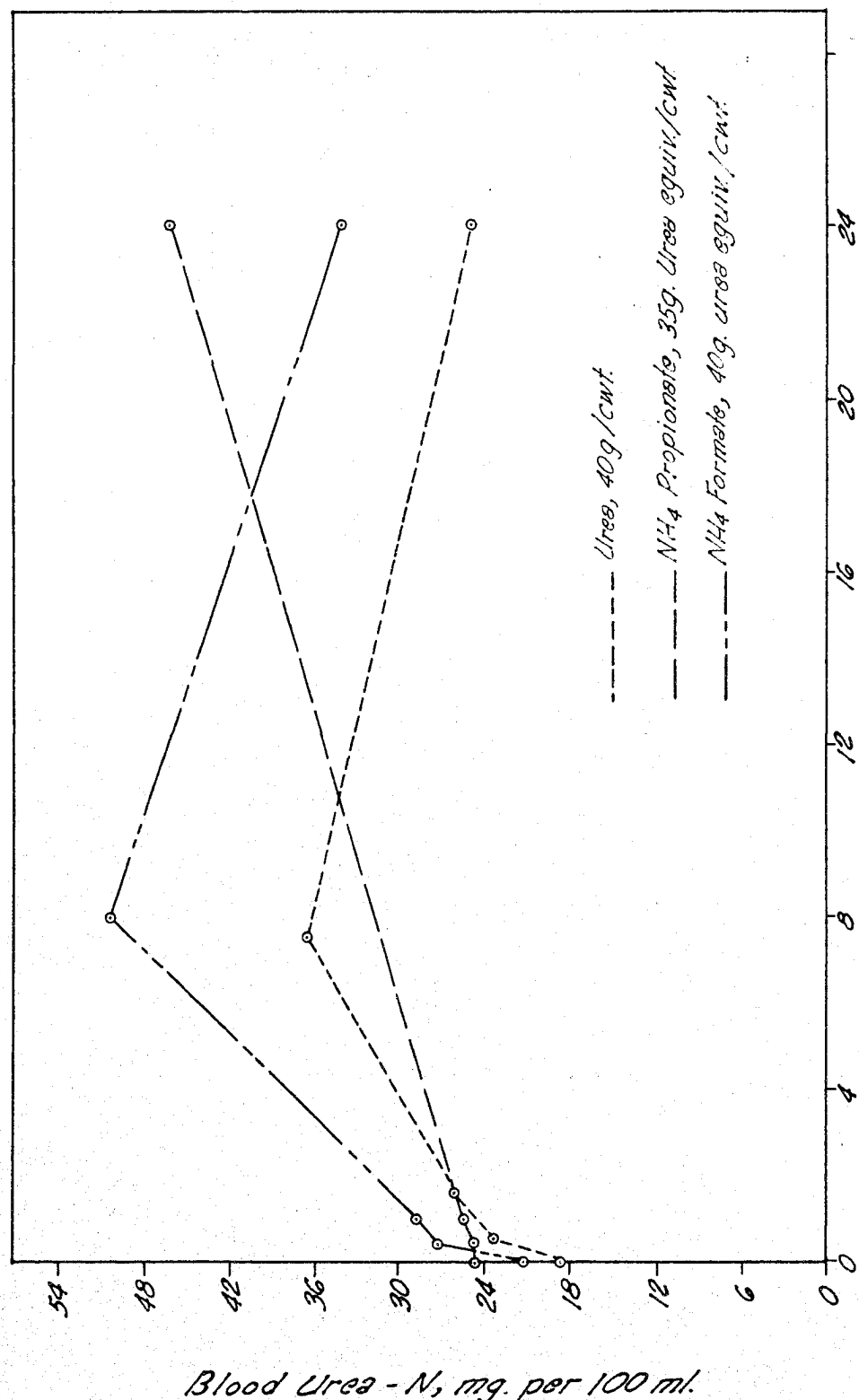
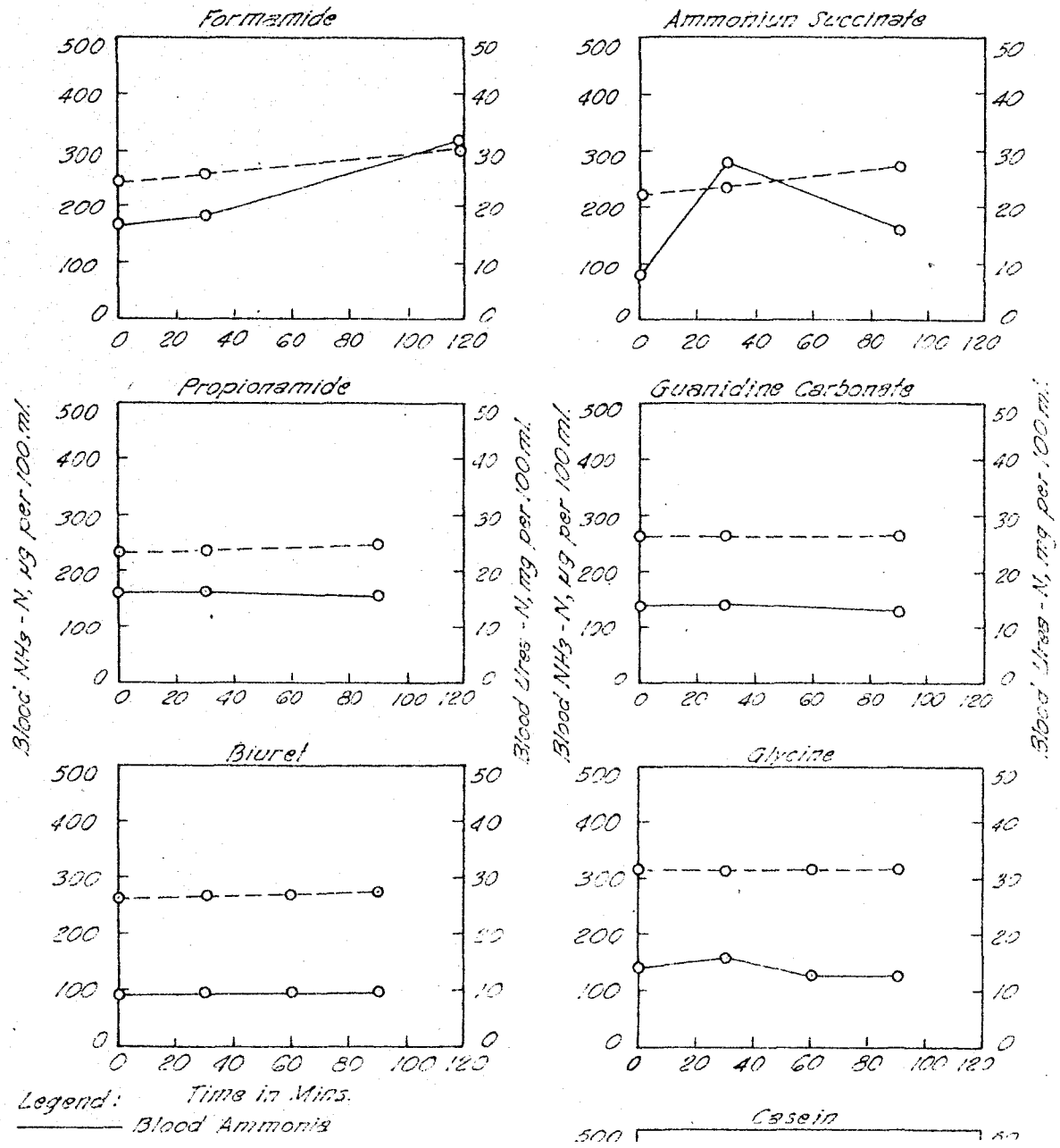


Figure 6. Influence of Administration of NPN Compounds on Blood Urea Values Over Extended Periods With Lambs

Table 6. Influence of oral administration of NPN compounds on blood urea-N values of lambs measured over extended periods (milligrams per 100 milliliters)

Bleeding intervals	Compounds administered		
	Urea	Ammonium propionate	Ammonium formate
0 time	18.93	24.31	21.28
+1/2 hr.	23.31	24.50	27.34
+1 hr.		25.52	28.55
+1 1/2 hrs.	26.29		
+7 1/2 hrs.	36.20		
+8 hrs.			50.57
+24 hrs.	24.81	46.20	34.14


Formamide
Ammonium Succinate
Propionamide
Guanidine Carbonate
Biuret
Glycine
Casein
Blood NH3-N, µg per 100 ml.
Blood Urea-N, mg per 100 ml.
0 20 40 60 80 100 120
0 100 200 300 400 500
0 10 20 30 40 50
Legend:
Time in Mins.
Blood Ammonia

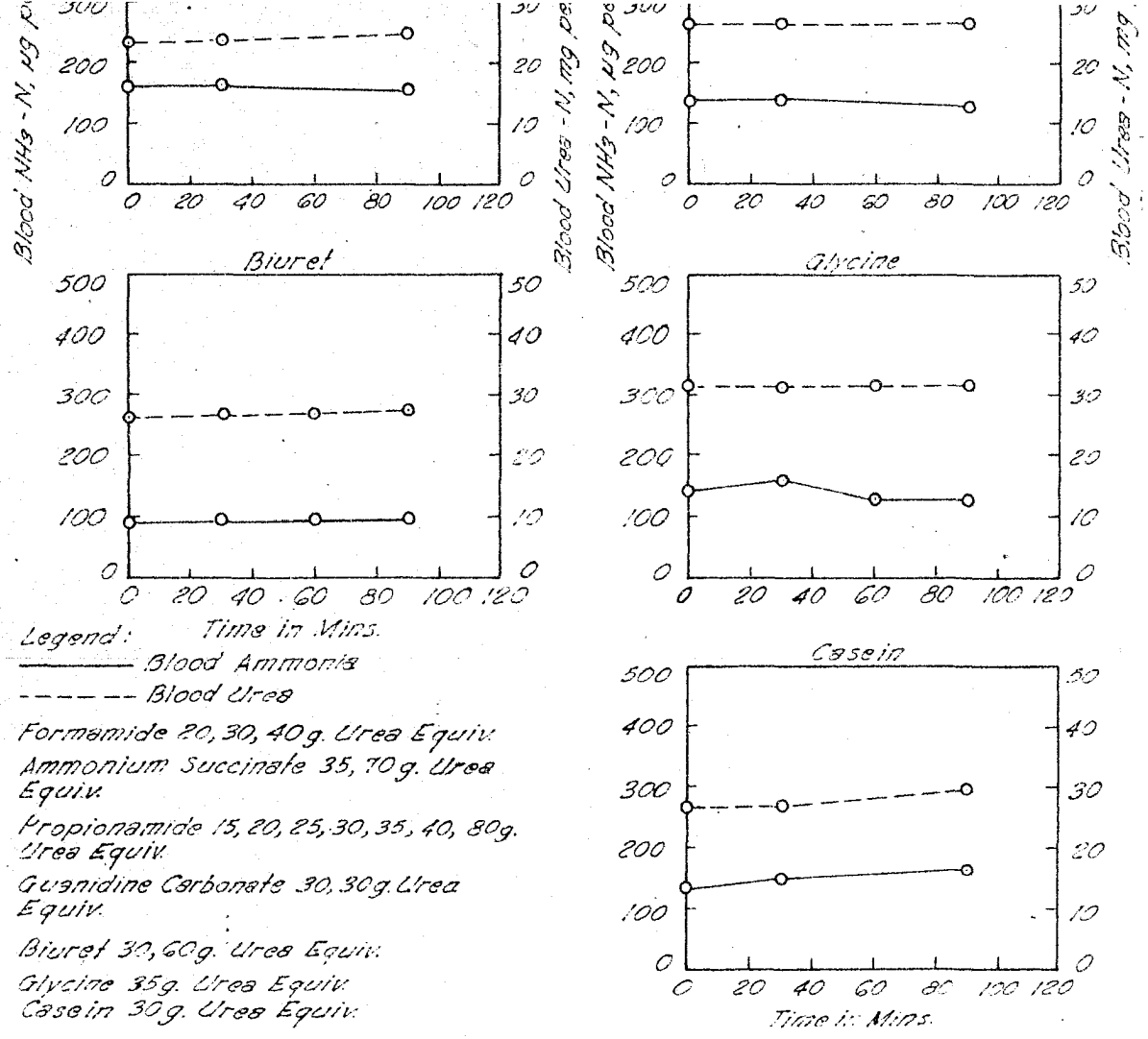


Figure 7. Observations on Substances not Found to Produce Ammonia Toxicity in Lambs

Table 7a. Blood NH_3-N following oral administration to lambs of substances not found to cause ammonia toxicity (gamma per 100 milliliters)

Bleeding intervals	Compounds administered							
	Formamide	Ammonium succinate	Guanidine carbonate	Biuret	Glycine	Casein	Propionamide	
0 time	167	115	111	74	146	136	106	206
	154	57	171	119			122	203
	186						110	185
							124	204
							190	129
							169	207
							129	199
+30 mins.	188	212	124	74	159	150	154	179
	189	346	164	113			140	171
	192						137	181
								167
								91
								202
								196
+60 mins.				74	127			
				119				
+90 mins.		266	102	74	126		120	234
		56	165	119		164	121	204
							114	
							125	
+120 mins.	330							
	315							
	321							

Table 7b. Blood urea-N following oral administration to lambs of substances not found to cause ammonia toxicity (milligrams per 100 milliliters)

Bleeding intervals	Compounds administered							
	Formamide	Ammonium succinate	Guanidine carbonate	Biuret	Glycine	Casein	Propionamide	
0 time	26.74	23.00	28.45	20.15	31.62	26.44	24.02	26.64
	21.60	21.32	25.16	33.65			28.84	20.59
	24.25						23.82	18.24
							22.88	20.62
							26.56	22.84
							26.09	23.38
							27.04	20.47
+30 mins.	27.40	23.50	28.30	20.15	31.21	27.02	28.06	26.55
	24.02	23.00	25.16	33.45			26.39	20.54
	26.52						28.30	18.46
								20.68
								22.78
								24.68
								20.37
+60 mins.				21.21	31.21			
				33.45				
+90 mins.		27.18	28.17	21.27	31.33	29.80	25.56	24.22
		27.10	25.20	34.60			29.82	20.51
							25.10	
							23.66	
+120 mins.	33.19							
	27.60							
	30.20							

Limited observations were made upon ammonium succinate, formamide, guanidine carbonate, biuret, casein and glycine. Based upon data collected for the first five compounds these compounds were given initially at or near a urea equivalent dose level that had previously produced toxicity. In the case of formamide three dose levels were used, two dose levels for guanidine carbonate, ammonium succinate and biuret with one dose level being used with casein and glycine. The results of drenching with these compounds on blood ammonia and urea levels are given in Figure 7 and Tables 7a and 7b. The average of dose levels are recorded as there appeared to be little difference due to level. No substantial rise was noted with any of these compounds suggesting that the ammonia was released by these compounds in the digestive tract slowly if at all. In no instance with these compounds did clinical symptoms of toxicity occur. However, with the guanidine carbonate both animals died the night following drenching. Eight hours after drenching these animals had appeared normal. Kutscher and Ackermann (23) reported on animal guanidine toxicity.

Clark et al. (8) reported that acetic acid when administered intravenously or orally with urea would prevent urea toxicity. In the present study it was found that acetic acid when administered simultaneously as a drench with levels of urea, ammonium formate and ammonium acetate which had

previously proven toxic caused distinctly lower blood ammonia values than when the non-protein nitrogen compounds were administered alone. Furthermore no clinical symptoms of toxicity occurred. The effect of the simultaneous dose of acetic acid and toxic nitrogen compounds upon blood ammonia values is shown in Figure 8 and Table 8. One normal acetic acid was administered on an equivalent urea ammonia molar basis. One lamb drenched with 80 grams of acetic acid along with a toxic dose of urea (40 grams) remained off feed for about 24 hours; however, no symptoms of urea toxicity appeared.

Attempts to alleviate urea toxicity once the symptoms appeared were not completely successful. Administration of acetic acid alleviated symptoms of toxicity in two animals that had been dosed with toxic levels of urea. One animal was exhibiting ataxia and the other had collapsed. However, toxicity could not be arrested if the animals were in a state of tetany. The results of these attempts to alleviate symptoms of toxicity are summarized in Figure 9.

Intravenous diffusions of 100 and 300 milliliters of 1 normal acetic acid immediately following drenching with a toxic level of urea did not prevent fatal toxicity. This is in contrast with the findings of Clark *et al.* (8) who prevented urea toxicity by intravenous administration of acetic acid although they did not state the amounts of

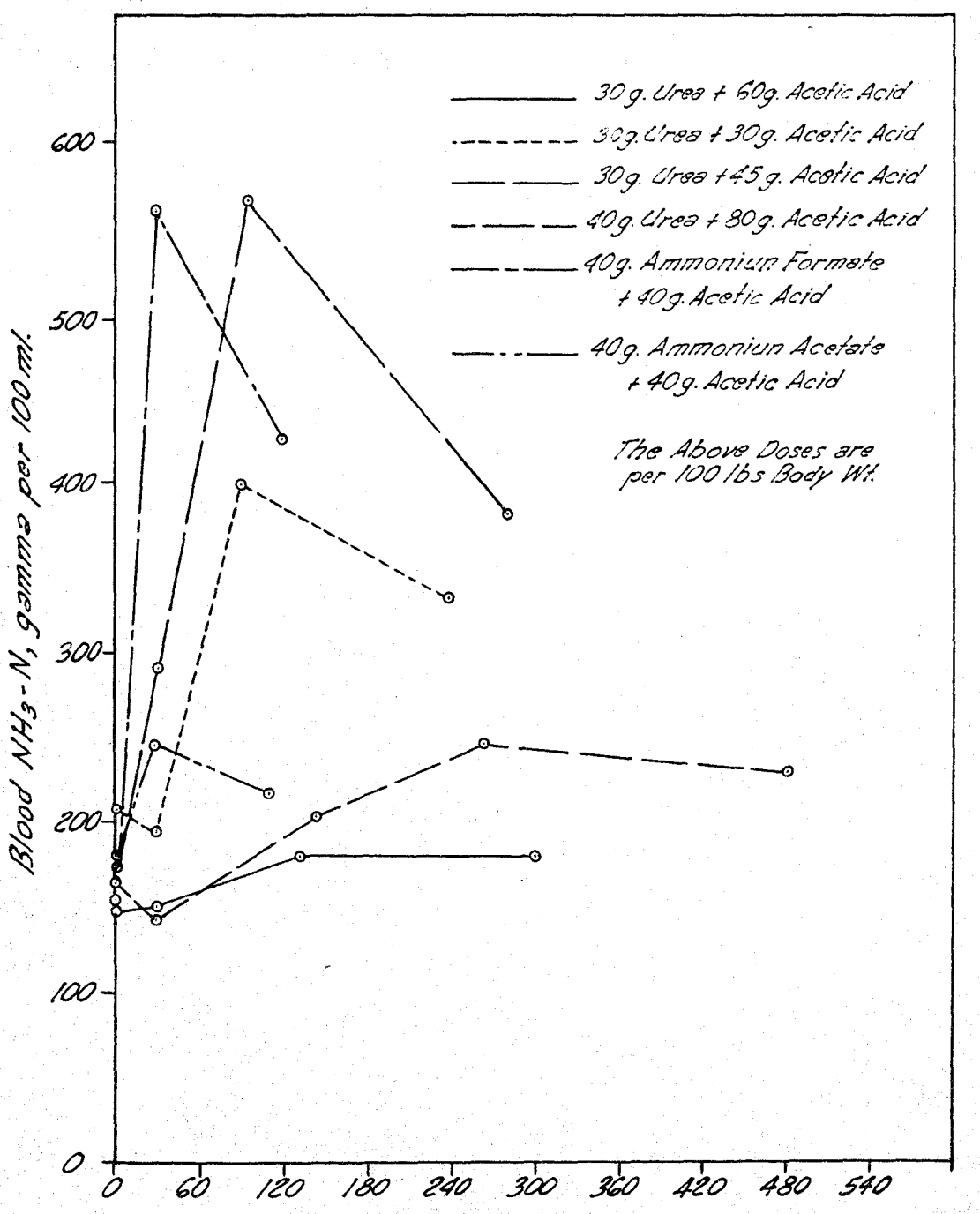


Figure 8. Blood NH_3-N Following Simultaneous Administration of NPN Compounds & Acetic Acid to Lambs

Table 8. Blood NH_3-N following simultaneous oral administration of NPN compounds and acetic acid to lambs (gamma per 100 milliliters)

Bleeding intervals	30g.urea + 30g.acetic acid	30g.urea + 45g.acetic acid	30g.urea + 60g.acetic acid	40g.urea + 80g.acetic acid	40g.ammonium formate + 40g.acetic acid	40g.ammonium acetate + 40g.acetic acid
0 time	206	172	147	167	157	179
+30 mins.	195	290	150	147	557	246
+90 mins.	400	568				
+108 mins.						217
+120 mins.					425	
+132 mins.			181			
+144 mins.				203		
+240 mins.	329					
+264 mins.				245		
+282 mins.		380				
+300 mins.			181			
+480 mins.				156		

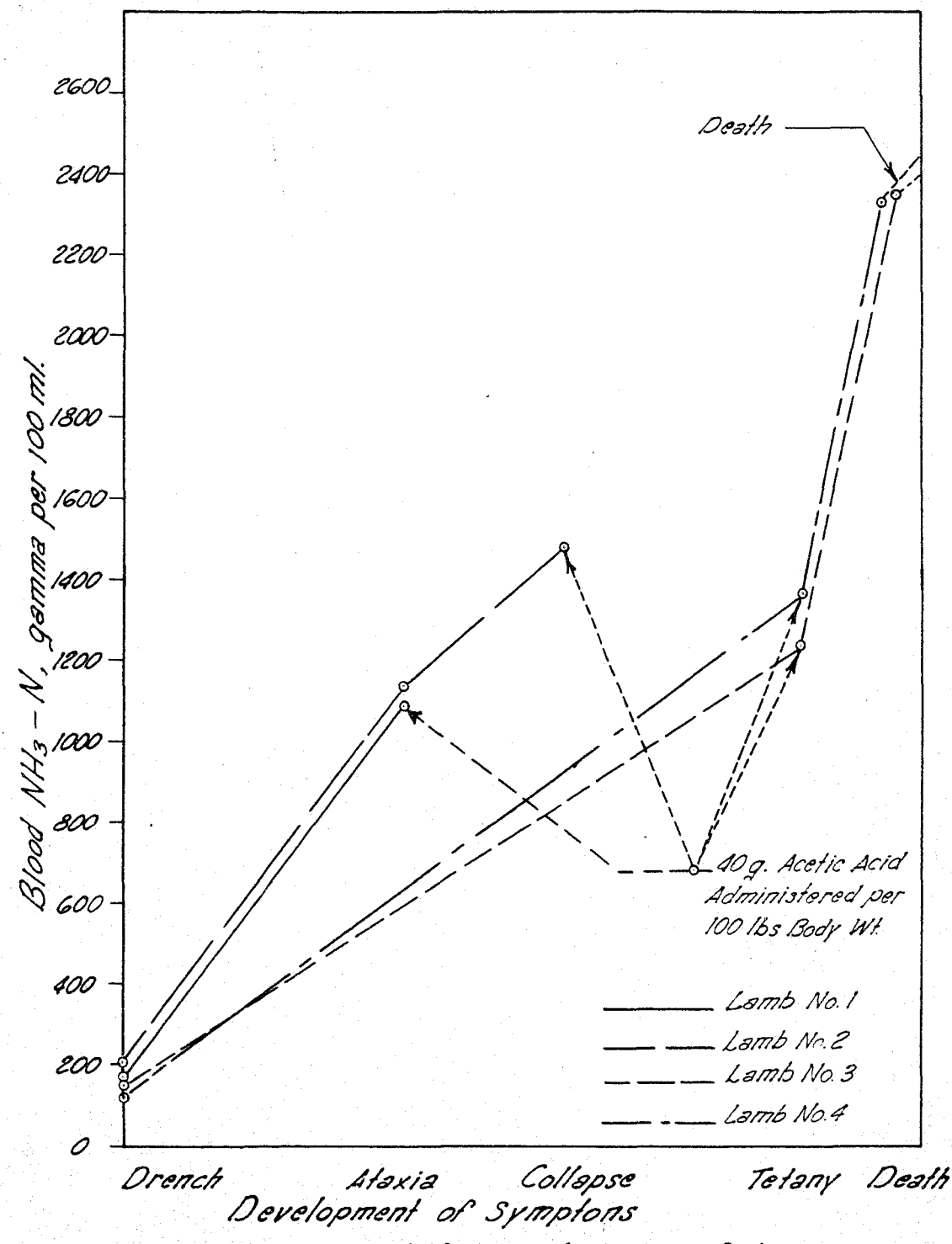


Figure 9. Oral Administration of Acetic Acid Subsequent to Drenching With Toxic Levels of Urea.

acetic acid used.

The effect of dosing with high levels of urea upon animals that had been held off feed for varying lengths of time was also checked. There were two animals per treatment except where fatal toxicity was encountered. Table 9 shows that withholding feed from one to 24 hours did not have any pronounced effect on blood NH_3-N levels. However, there was some suggestion that after a 24-hour fast the high dose levels of urea were somewhat more toxic.

Table 9. The effect of administration of urea at various time intervals after feeding upon maximum blood ammonia values of lambs (gamma per 100 milliliters)

Dose level (gms. urea/ 100 lbs. wt.)	Time of administration after feeding		
	1 hour	18 hours	24 hours
30	154	626	384
35	827	800*	1095**
40	Fatal toxicity	Fatal toxicity	

*Outward symptoms of toxicity in one of two animals represented (animal recovered).
**Outward symptoms of toxicity in this animal (acetic acid administered to prevent fatal toxicity).

In an additional study blood samples were taken from four lambs after they had been drenched with propionamide. Two of these lambs received 40 grams urea equivalent and two received 80 grams urea equivalent of propionamide. All four lambs treated in this study had been receiving propionamide in their ration at a level of 45 per cent of the protein equivalent for 49 days. The purpose of this part of the study was to determine whether or not ammonia was released more rapidly into the blood of these lambs as a result of their having received propionamide in their diet. Tables 10 and 11 reveal that at the 40 gram level of administration there was only a slight increase in blood ammonia and urea levels. However, at the 80 gram level of administration there was an appreciable increase in blood ammonia and urea levels 30 minutes after drenching. These results differ from those recorded when propionamide was administered to lambs that had not received propionamide in the ration, in that in these instances essentially no increase in either blood ammonia or urea were found.

Results presented above show blood ammonia to be a rather critical measurement of the toxicity of NPN compounds. As dose levels of the toxic compounds were increased a rise was noted in blood ammonia. Analysis of the data show a correlation coefficient of 0.94 indicating a high degree of association between blood ammonia values and the dose level

Table 10. Blood NH_3-N of lambs drenched with propionamide after having received it in the ration for 49 days (gamma per 100 milliliters)

Lamb no.	Bleeding interval	Dose level	Blood NH_3-N
148	0 time	40 g./cwt.	120
"	+30 mins.	" "	137
149	0 time	" "	112
"	+30 mins.	" "	161
140	0 time	80 g./cwt.	140
"	+30 mins.	" "	316
122	0 time	" "	196
"	+30 mins.	" "	382

Table 11. Blood urea-N of lambs drenched with propionamide after having received it in the ration for 49 days (milligrams per 100 milliliters)

Lamb no.	Bleeding interval	Dose level	Blood urea-N
148	0 time	40 g./cwt.	14.00
"	+30 mins.	" "	14.92
149	0 time	" "	15.51
"	+30 mins.	" "	16.91
140	0 time	80 g./cwt.	19.27
"	+30 mins.	" "	23.04
122	0 time	" "	21.62
"	+30 mins.	" "	25.41

of the toxic compounds. This information suggests that the animals actually succumb to a toxicity resulting from ammonia. The most toxic compounds were urea, ammonium formate, ammonium propionate and ammonium acetate. These four compounds were nearly equivalent in toxicity when considered on an equal nitrogen basis with perhaps ammonium propionate being the most toxic of the four. The toxic level of these four compounds when administered as a single drench was about 40 grams of urea equivalent per 100 pounds of body weight.

The lambs in this study appeared to be able to stand blood NH_3-N values of about 1000 micrograms per 100 milliliters of whole blood without showing outward symptoms. Normal blood NH_3-N levels were between 80 and 250 micrograms (average, 148). If blood NH_3-N values went no higher than 1150 micrograms per 100 milliliters nearly all the animals survived. Just prior to death blood NH_3-N values of 2 to 3.5 milligrams per cent were recorded. These observations indicate that the critical range of blood ammonia has rather narrow limits. The NH_3-N values reported in this thesis are considerably lower than those reported by Dinning *et al.* (10). It should be pointed out that the microdiffusion technique used in this experiment is much more sensitive for blood NH_3-N than the older areation methods.

When large doses of the toxic non-protein nitrogen compounds were given it was possible to measure an increase in the blood ammonia 15 minutes after dosing. This indicated an extremely rapid liberation of the ammonia from the non-protein nitrogen compounds. The first clinical symptoms appeared at about 30 to 45 minutes after dosing. The animals at this time became restless and shortly afterward exhibited ataxia. These symptoms were usually of 10 to 15 minutes duration after which the animals collapsed. During this stage of toxicity labored breathing was encountered, accompanied by frothing at the mouth. Bloating sometimes occurred during this period suggesting a cessation of rumen motility. Usually about 25 minutes after collapse the animals went into tetany. Labored breathing continued and bloating was obvious. The skin of the animals took on a blue color which suggested anoxia. Death usually occurred one and one-half to two hours after dosing. The most consistent findings upon necropsy of animals succumbing to toxic doses of the non-protein nitrogen compounds are described below. Extensive epicardial and endocardial hemorrhages were nearly always found. The blood vessels were usually cyanotic. A swollen and congested condition was present in most of the kidneys examined. A brownish discoloration of the blood was prevalent in many of the animals suggesting methemoglobin formation. Some

hemorrhagic conditions were noted in the abomasum and intestinal tract. The clinical symptoms of toxicity and necropsy findings are similar to those reported by Clark et al. (8).

Several of the compounds tested appeared to be non-toxic in respect to rapid liberation of ammonia. The ammonium salts of the organic acids were toxic with one exception. Ammonium succinate proved to be non-toxic. At present the reason for its low toxicity is unknown. The amides (propionamide, formamide, biuret) were non-toxic. This is presumably due to a lack of amidases in the rumen. The administration of propionamide at high levels to lambs already receiving this compound in the ration resulted in moderate increases in blood ammonia and urea, suggesting an adaptation of the rumen microorganisms to the release of the ammonia nitrogen. This suggestion was confirmed by later experiments reported in this thesis. Urea which is a diamide of carbonic acid was highly toxic. However, this may be explained on the basis of the high urease activity of the rumen contents which readily liberates the urea ammonia. The amidine guanidine carbonate was not toxic in respect to ammonia toxicity but did cause death of the animals. This was apparently a specific toxicity of guanidine rather than ammonia toxicity. The amine glycine was non-toxic. Belasco (1) has shown that the nitrogen of glycine is not available

to rumen microorganisms. Hoflund et al. (18) reported that a sheep which had been on a poor diet died following administration of casein. In the study reported in this thesis, however, no rise in blood ammonia was noted when casein was administered at a 30 gram urea equivalent dose level. The nutrition of this lamb was good, although it had been withheld from feed 18 hours prior to drenching.

Summary

Ten non-protein nitrogen compounds were administered orally to lambs for purposes of determining their toxicity. These compounds were administered in progressively larger doses and blood samples were taken at varying intervals following administration. Ammonia and urea nitrogen were determined on 350 blood samples taken from 30 lambs in studying the toxicity of urea, ammonium formate, ammonium acetate, ammonium propionate and propionamide. Limited observations were made upon 15 lambs using ammonium succinate, formamide, guanidine carbonate, biuret and glycine. In all cases except with guanidine carbonate, toxicity was associated with large increases in blood ammonia nitrogen, with the critical level being about 1 milligram per 100 milliliters of blood. Administration of urea, ammonium formate, ammonium acetate and ammonium propionate at a level of about

40 grams urea equivalent resulted in fatal toxicity. Fatal toxicity not associated with an increase in blood ammonia nitrogen was observed after administration of guanidine carbonate at the 30 gram urea equivalent level. Little if any increase in blood ammonia and urea nitrogen attended the administration of the other compounds except where large doses of propionamide were administered to lambs receiving this compound in the ration in which case moderate increases in blood ammonia and urea nitrogen resulted. Symptoms of fatal toxicity were alleviated by oral administration of adequate amounts of acetic acid.

Value of Several Non-protein Nitrogen Compounds as Protein Substitutes in Lamb Fattening Rations

Part I. Group Feeding Trials

Several non-protein nitrogen compounds other than urea have been used as nitrogen sources in the nutrition of ruminants. Of all non-protein nitrogen compounds urea has been used by far the most widely as a nitrogen source in ruminant rations. It seemed desirable to compare the efficiency of urea and several other non-protein nitrogen compounds as protein substitutes as well as to compare these compounds with protein from conventional sources, since the experimental data from these types of comparisons are

scarce. It is believed that if competition between ruminants and non-ruminants for protein concentrates becomes more intense, information as to the comparative efficiency of non-protein nitrogen compounds as protein substitutes will become more valuable.

Materials and methods

This study consisted of two group feeding experiments. In Experiment I, 50 feeder lambs averaging about 75 pounds in weight were allotted into five lots of ten lambs each. The experimental rations appear in Table 12. At the start of the test period each lamb received two pounds of corn silage plus one pound of the experimental ration daily. The amount of corn silage was decreased and the amount of experimental ration increased at two or three-day intervals so that by the fourteenth day of the test the lambs were receiving the experimental ration alone. The five non-protein nitrogen compounds fed in this trial were urea, ammonium propionate, formamide, propionamide and ammonium formate. Experiment II included all the compounds tested in Experiment I except that ammonium acetate was substituted for formamide. The ammonium acetate lots were replicated in Experiment II since no previous feeding trial had been conducted with this compound. In addition positive and

Table 12. Experimental rations group fed to lambs in Experiment I (expressed in pounds)

	Urea	Ammonium propionate	Formamide	Propionamide	Ammonium formate
Corn cobs	20.000	20.000	20.000	20.000	20.000
Clover hay	10.000	10.000	10.000	10.000	10.000
Corn (cracked)	40.000	40.000	40.000	40.000	40.000
Molasses (cane)	25.928	25.928	25.928	25.928	25.928
Live yeast	.002	.002	.002	.002	.002
Mono-calcium phosphate	.800	.800	.800	.800	.800
Limestone	.800	.800	.800	.800	.800
Salt (iodized)	.150	.150	.150	.150	.150
Trace minerals	.080	.080	.080	.080	.080
Vitamin A and D oil	.040	.040	.040	.040	.040
Urea	2.200	--	--	--	--
Ammonium propionate	--	8.234	--	--	--
Formamide	--	--	3.014	--	--
Propionamide	--	--	--	4.803	--
Ammonium formate	--	--	--	--	4.154
Total	100.000	106.034	100.814	102.603	101.954
Per cent protein	11.61	11.59	11.64	11.61	11.63

negative control ots were included (Table 13). Twenty lambs averaging about 70 pounds in weight were fed per lot in Experiment II. These animals were brought on feed slowly, but no corn silage was fed as in Experiment I. In both experiments the non-protein nitrogen compounds furnished 50 per cent of the dietary nitrogen. Fourteen-day individual weights were taken for lambs in both experiments.

Results and discussion

The results of Experiments I and II are summarized in Tables 14 and 15. The difference in daily gain between the lambs receiving urea and those receiving ammonium propionate in Experiment I was significant ($P < .05$), and the difference in gain between the urea lambs and those receiving formamide was highly significant ($P < .01$). Differences in daily gain between the lambs receiving urea and those receiving ammonium formate and propionamide were not statistically significant. It will be noted in Table 14 that the lambs fed the urea and ammonium formate rations consumed the most feed, 3.61 pounds daily in each case. The lambs receiving formamide consumed less feed than lambs on any other treatment. Although some difficulty was experienced in keeping the ammonium propionate lambs on full-feed, all other lots consumed progressively more feed during the

Table 13. Experimental rations group fed to lambs in Experiment II[a] (expressed in pounds)

	Conventional ration	Low nitrogen ration	Urea	Ammonium propionate	Propionamide	Ammonium formate	Ammonium acetate
Ground corn cobs	20.00	20.00	20.00	20.00	20.00	20.00	20.00
Clover hay	10.00	10.00	10.00	10.00	10.00	10.00	10.00
Cane molasses	25.00	25.00	25.00	25.00	25.00	25.00	25.00
Cracked shelled corn	27.65	42.90	40.70	33.20	36.83	38.27	37.14
Mono-calcium phosphate	.75	1.00	1.00	1.00	1.00	1.00	1.00
Salt (iodized)	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Trace minerals	.10	.10	.10	.10	.10	.10	.10
Soybean oil meal	15.50	--	--	1.50	.75	.50	.75
Urea	--	--	2.20	--	--	--	--
Ammonium propionate	--	--	--	8.20	--	--	--
Propionamide	--	--	--	--	5.32	--	--
Ammonium formate	--	--	--	--	--	4.13	--
Ammonium acetate	--	--	--	--	--	--	5.00
Total	100.00	100.00	100.00	100.00	100.00	100.00	100.00
Per cent protein	11.58	6.07	11.64	11.64	11.63	11.63	11.60
Per cent NPN	--	--	5.76	5.74	5.75	5.75	5.70

[a]50 milliliters vitamin A and D oil added to each ration.

Table 14. Effect of group feeding non-protein nitrogen compounds upon growth, feed consumption and feed efficiency of lambs - Experiment I

		Urea	Ammonium propionate	Formamide	Propionamide	Ammonium formate
Av. initial weight	lb.	74.4	73.0	74.6	73.7	73.4
Av. final weight	lb.	96.0	85.4	80.9	88.6	97.0
Gain per lamb	lb.	21.6	12.4	6.3	14.9	23.6
Daily gain per lamb	lb.	.39	.22	.11	.27	.42
Daily feed consumption[a]	lb.	3.61	2.66	2.38	3.08	3.61
Feed per pound gain	lb.	9.35	12.0	21.04	11.53	8.55
Initial number of lambs		10[b]	10	10[b]	10[c]	10

[a]These amounts include about .25 pound corn silage.
[b]One lamb removed from lot due to sickness; estimated feed consumed charged to the treatment.
[c]Two lambs died late in experiment; weight gains and estimated feed consumption calculated to the weigh day preceding death.

Table 15. Effect of group feeding non-protein nitrogen compounds upon growth, feed consumption and feed efficiency of lambs[a] - Experiment II

		Conventional ration	Low nitrogen ration	Urea	Ammonium propionate	Ammonium acetate	Propionamide	Ammonium formate
Av. initial wt.	lb.	73.9	74.7	77.6	77.0	77.8	77.0	76.1
Av. final wt.	lb.	103.4	85.2	94.8	99.8	100.6	98.2	99.5
Gain per lamb	lb.	29.5	10.5	17.2	22.8	22.8	21.2	23.4
Daily gain per lamb	lb.	.40	.14	.23	.31	.31	.29	.31
Feed consumption per lamb	lb.	3.54	2.78	3.15	3.32	3.37	3.28	3.40
Feed per pound gain	lb.	8.64	19.14	13.16	10.61	10.64	11.13	10.76
Initial number of lambs		20	20	20	20[a]	40	20	20

[a]One lamb died during the experiment; weight gains and estimated feed consumption calculated to the weigh day preceding death.

trial. The feed efficiency data from Experiment I indicates that greater feed efficiency was directly associated with body weight gains. The lambs receiving ammonium formate showed the greatest feed efficiency with the urea-fed lambs next.

In Experiment II the daily gains for the lambs receiving the conventional protein ration were significantly higher ($P < .05$) than for the lambs receiving ammonium acetate and propionamide, and were markedly higher ($P < .01$) than for the lambs receiving urea. No statistically significant difference existed between the gains of the conventional protein-fed lambs and those receiving either ammonium formate or ammonium propionate. It was found that the gains of all lambs except those receiving urea were considerably higher ($P < .01$) than the gains of the low nitrogen negative control lambs. The data would suggest that except in the case of urea the nitrogen of the non-protein nitrogen compounds was utilized in growth and fattening although not to the extent of the nitrogen from natural sources. It should be explained that the average weight gains of the urea lambs was reduced by three lambs on this treatment one of which failed to gain and two of which lost weight steadily during the trial. It was questionable whether the response of these three lambs was due to treatment. Although significant differences in weight gains existed between the control

lambs (positive and negative) and some of the other rations as indicated above, no significant differences in weight gains existed among lambs receiving the non-protein nitrogen compounds in Experiment II. As in Experiment I, feed efficiency was closely associated with weight gain, with the greatest feed efficiency being recorded for the lambs on the conventional protein ration and the lowest by the negative control lambs. The feed efficiency among the lambs receiving the non-protein nitrogen compounds was quite similar with the exception of the urea lambs for which the feed efficiency was somewhat lower than the rest.

From the results of the first feeding experiment formamide was eliminated from consideration in further group feeding trials since the lambs appeared not to relish this compound and gained poorly on it. Although the weight gains for the lambs receiving ammonium propionate were significantly lower than for the urea control lambs it was decided to include this compound in the second feeding experiment. Results of the second feeding experiment indicated no statistically significant differences among weight gains for lambs fed ammonium formate, propionamide, ammonium acetate, ammonium propionate or urea, although significant differences in weight gains were found to exist between the lambs receiving conventional protein and those receiving urea, propionamide and ammonium acetate. It appeared from

these two experiments that several non-protein nitrogen compounds might be equally efficient as protein substitutes in lamb fattening rations. Toxicity studies conducted concurrently with these feeding tests modified this conclusion.

Summary

Two feeding experiments were conducted with fattening lambs in which 50 per cent of the protein equivalent of the rations was furnished by urea, ammonium acetate, ammonium propionate, ammonium formate, formamide or propionamide. Formamide was found to be inferior to the other compounds tested as measured by weight gains. In one experiment ammonium propionate supported significantly smaller weight gains than did the urea control ration, but in a second experiment this compound was equal to the other non-protein nitrogen compounds tested. With the exceptions indicated above there were no great differences in weight gains among lambs receiving any of the compounds. The lambs receiving rations in which protein came entirely from natural sources gained more rapidly than did lambs receiving non-protein nitrogen compounds.

Value of Several Non-protein Nitrogen Compounds as Protein Substitutes in Lamb Fattening Rations

Part II. Individual Feeding Trial

In the group feeding trials preceding this experiment comparisons were made between lamb rations in which all the protein was furnished from natural sources and rations in which one-half the protein equivalent was furnished by one of several non-protein nitrogen compounds. In these trials it was found that weight gains among lambs receiving the non-protein nitrogen compounds were in most instances nearly as high as the weight gains of lambs receiving the conventional ration except during the first two or three weeks of the feeding period. It appeared that the lambs required a two or three week period to adapt themselves to the non-protein nitrogen compounds. It seemed desirable to determine whether this adjustment period could either be reduced or eliminated by reducing the proportion of nitrogen furnished by the non-protein nitrogen compounds. On the basis of favorable results with propionamide in the previous feeding trials and especially because of its non-toxicity this compound was selected for use in this individual lamb feeding trial with urea being fed at identical levels as a control compound.

Materials and methods

Forty-three lambs averaging between 75 and 80 pounds in weight were obtained for this experiment. The lambs were vaccinated for enterotoxemia prior to the start of the experiment and were fed clover hay, shelled corn and soybean oil meal for a period of about two weeks before the experiment actually started on July 8, 1954. The lambs were randomly allotted to treatment and to feeding stalls. All lambs were individually fed and all treatments were replicated. Urea and propionamide were each fed at two identical levels on a nitrogen basis (15 and 30 per cent of the protein equivalent of the ration). Positive and negative control rations were also included. The experimental rations appear in Table 16. Individual feed consumption and individual 14-day body weights were recorded.

Results and discussion

The results of the experiment are summarized in Table 17. The differences in rate of gain among lambs in this experiment were analyzed statistically with the following results:

Propionamide versus Urea - Not significant
Conventional versus Propionamide (both levels) - Not significant

Table 16. Experimental rations individually fed to lambs[a] (expressed in pounds)

	Low nitrogen ration	30% of protein from urea	15% of protein from urea	30% of protein from propion-amide	15% of protein from propion-amide	100% of protein from natural sources
Ground corn cobs	16.00	16.00	16.00	16.00	16.00	16.00
Clover-timothy hay	20.00	20.00	20.00	20.00	20.00	20.00
Cane molasses	19.00	19.00	18.00	17.00	17.00	17.00
Cracked shelled corn	42.20	40.75	38.35	41.33	38.64	35.95
Soybean oil meal	1.00	1.25	5.25	1.25	5.25	9.25
Iodized salt	1.00	1.00	1.00	1.00	1.00	1.00
Mono-calcium phosphate	.80	.80	.80	.80	.80	.80
Urea	--	1.20	.60	--	--	--
Propionamide	--	--	--	2.62	1.31	--
Total	100.00	100.00	100.00	100.00	100.00	100.00
Per cent protein	7.38	10.51	10.51	10.51	10.51	10.52

[a] 10 grams of vitamin A concentrate (containing 5,000 International Units of vitamin A acetate per gram) added per 100 pounds of ration.

Table 17. Effect of individual feeding of urea and propionamide upon growth, feed consumption and feed efficiency of lambs

Conventional ration										
Lamb no.		11	21	31	41	51	61	71	81	Average
Initial wt.	lb.	91	86	83	81.5	81	82	81	77.5	82.9
Final wt.	lb.	120.5	123.5	109.5	107.5	102.5	116	111	105	111.9
Gain per lamb	lb.	29.5	37.5	26.5	26	21.5	34	30	27.5	29.0
Daily gain per lamb	lb.	.53	.65	.47	.46	.38	.61	.54	.49	.52
Feed consumption per lamb	lb.	4.21	4.42	3.67	3.77	3.35	4.27	3.68	3.83	3.90
Feed per pound gain	lb.	8.00	6.60	7.75	8.12	8.72	7.03	6.87	7.80	7.51
Low nitrogen ration										
Lamb no.		12	22	32	42	52	62	72		
Initial wt.	lb.	89.5	86.5	83	83.5	79.5	75.5	78		82.4
Final wt.	lb.	115	109	113	101.5	98	91.5	92		102.9
Gain per lamb	lb.	25.5	22.5	30	18	18.5	16	14		20.5
Daily gain per lamb	lb.	.46	.40	.54	.32	.33	.29	.25		.37
Feed consumption per lamb	lb.	4.21	3.42	4.04	3.46	3.09	3.45	3.56		3.61
Feed per pound gain	lb.	9.25	8.51	7.55	10.78	9.35	12.06	14.25		9.88

Table 17. (Continued)

Propionamide, 15 per cent of protein equivalent									
Lamb no.		13	23	33	43	53	63	73	Average
Initial wt.	lb.	88	84	83.5	81	76.5	76	78	81
Final wt.	lb.	118	107	110	106	98.5	99	100.5	105.6
Gain per lamb	lb.	30	23	26.5	25	22	23	22.5	24.6
Daily gain per lamb	lb.	.54	.41	.47	.45	.39	.41	.40	.44
Feed consumption per lamb	lb.	4.31	3.62	3.90	4.08	3.32	3.33	3.49	3.72
Feed per pound gain	lb.	8.05	8.80	8.25	9.14	8.45	8.11	8.69	8.48
Propionamide, 30 per cent of protein equivalent									
Lamb no.		14	24	34	44	54	64	74	
Initial wt.	lb.	92.5	88	85.5	84	78	81.5	77	83.8
Final wt.	lb.	124	121.5	120	106	100.5	112	99	111.9
Gain per lamb	lb.	31.5	33.5	34.5	22	22.5	30.5	22	28.1
Daily gain per lamb	lb.	.56	.60	.62	.39	.40	.54	.39	.50
Feed consumption per lamb	lb.	3.97	4.33	4.20	3.72	4.10	4.32	3.35	4.00
Feed per pound gain	lb.	7.06	7.24	6.81	9.48	10.20	7.93	8.52	7.98

Table 17. (Continued)

		Urea, 15 per cent of protein equivalent							
Lamb no.		15	25	35	45	55	65	75	Average
Initial wt.	lb.	90.5	84.5	81	80.5	81.5	80.5	74.5	81.9
Final wt.	lb.	116	105	109.5	110	104.5	102	94	105.9
Gain per lamb	lb.	25.5	20.5	28.5	29.5	23	21.5	19.5	24
Daily gain per lamb	lb.	.46	.37	.51	.53	.41	.38	.35	.43
Feed consumption per lamb	lb.	4.11	3.56	4.21	4.25	3.27	3.38	3.21	3.71
Feed per pound gain	lb.	9.02	9.73	8.26	8.07	7.96	8.79	9.23	8.66
		Urea, 30 per cent of protein equivalent							
Lamb no.		16	26	36	46	56	66	76	Average
Initial wt.	lb.	86.5	90	81	84.5	80	79	76.5	82.5
Final wt.	lb.	121	108.5	95	114	105	110.5	100.5	107.8
Gain per lamb	lb.	34.5	18.5	14	29.5	25	31.5	24	25.3
Daily gain per lamb	lb.	.62	.33	.25	.53	.45	.56	.43	.45
Feed consumption per lamb	lb.	4.40	3.55	3.99	4.46	4.34	4.59	3.42	4.11
Feed per pound gain	lb.	7.14	10.76	15.96	8.46	9.72	8.16	7.98	9.10

Conventional versus Urea (both levels) - Not significant
Conventional versus Low Nitrogen - ($P < .01$)
Low nitrogen versus Propionamide (both levels) - ($P < .05$)
Low nitrogen versus Urea (both levels) - Not significant
Differences due to levels for NPN compound - Not significant

From an examination of Table 17 and of the statistical results it may be seen that the differences in rate of gain between the lambs fed the conventional ration and those fed urea or propionamide were not statistically significant. Neither were the differences in gain significant between lambs fed propionamide and those fed urea, although the actual gains favored the propionamide-fed lambs. The differences in gain between the lambs receiving the conventional ration and those receiving low nitrogen ration were highly significant ($P < .01$). In comparing the rates of gain of the lambs fed the low nitrogen ration with the lambs fed propionamide it was found that the differences were significant ($P < .05$). No statistical significance was found to exist between gains of lambs fed urea and those fed the low nitrogen ration. It was also found that no statistically significant difference existed in rates of gain of lambs fed either non-protein nitrogen compound at the 15 per cent level as compared to the 30 per cent level.

It is believed that the primary reason that the lambs fed the non-protein nitrogen compounds in this experiment

compared so favorably in weight gains with the lambs receiving the conventional ration was because they gained well during the first two weeks of the experiment and did not appear to require a period for adaptation to the compounds. Another reason was that a small amount of soybean oil meal was included in each of the non-protein nitrogen rations. The meal is believed to have furnished growth factors stimulatory to the rumen bacteria.

Under the conditions of this experiment, therefore, the non-toxic non-protein nitrogen compound propionamide when fed at the moderate levels of 15 and 30 per cent of the protein equivalent supported weight gains in lambs slightly superior to urea and essentially as high as the conventional protein ration.

High feed consumption and feed efficiency were directly associated with high rate of gain except in the case of the 30 per cent level of urea. In this case although feed consumption was high feed efficiency was comparatively low. With the low nitrogen ration feed consumption was good but feed efficiency was comparatively poor. The feed efficiency on the conventional ration and 30 per cent level of propionamide were considered very good.

Summary

Forty-three lambs were individually fed rations containing urea and propionamide at levels of 15 and 30 per cent of the protein equivalent for 56 days. The lambs fed the conventional ration gained an average of .52 pounds per day. The lambs fed the 30 per cent level of propionamide gained essentially as well as the lambs fed the conventional ration. The lambs on the other rations gained as follows: 15 per cent level of propionamide, .44; 15 per cent level of urea, .43; 30 per cent level of urea, .45; and the low nitrogen negative control, .37 pounds per day.

Value of Several Non-protein Nitrogen Compounds as Protein Substitutes in Lamb Fattening Rations

Part III. Nitrogen Balance Study

The primary objective of this phase of the research was to evaluate growth data by doing a nitrogen balance study on several lambs. A secondary objective was to extend the previous study of the usefulness of several non-protein nitrogen compounds as substitutes for conventional protein in lamb fattening rations. The compounds studied were urea, ammonium propionate, formamide, propionamide and ammonium formate.

Materials and methods

Thirty-five western wether lambs averaging about 65 pounds in weight were obtained as experimental animals in January, 1953. They were weighed, ear tagged and vaccinated against enterotoxemia prior to the beginning of the experiment. The lambs were allotted to pens according to weight and randomly assigned to individual feeding stalls.

The rations fed are listed in Table 18. In the rations containing the non-protein nitrogen compounds, these compounds furnished 50 per cent of the dietary nitrogen. Two control rations were used, one a conventional lamb fattening ration and the other a ration similar to those containing the non-protein nitrogen compounds except that the non-protein nitrogen compounds were deleted.

The lambs were placed on the experimental rations early in February, 1953. After two weeks one animal from each of the seven ration groups was removed from the pen and placed in a metabolism cage. These seven lambs were then fed for a preliminary period of three days at slightly reduced intakes of feed. A seven-day collection period followed the three-day preliminary period. Feces and urine were collected and subsequently analyzed for nitrogen.

At the end of the seven-day collection period the seven lambs were returned to their respective pens and seven more

Table 18. Experimental rations with non-protein nitrogen compounds fed to lambs (expressed in pounds)

	Conventional ration	NPN deleted	Urea	Ammonium propionate	Formamide	Propionamide	Ammonium formate
Ground corn cobs	--	20.410	20.000	20.000	20.000	20.000	20.000
Clover hay	50.000	10.200	10.000	10.000	10.000	10.000	10.000
Corn (cracked)	32.128	40.988	40.000	40.000	40.000	40.000	40.000
Molasses (cane)	10.000	26.530	25.928	25.928	25.928	25.928	25.928
S.B.O.M.	6.000	--	--	--	--	--	--
Live yeast	.002	.002	.002	.002	.002	.002	.002
Mono-calcium phosphate	.800	.800	.800	.800	.800	.800	.800
Limestone	.800	.800	.800	.800	.800	.800	.800
Salt (iodized)	.150	.150	.150	.150	.150	.150	.150
Trace minerals	.080	.080	.080	.080	.080	.080	.080
Vitamin A and D oil	.040	.040	.040	.040	.040	.040	.040
Urea	--	--	2.200	--	--	--	--
Ammonium propionate	--	--	--	8.234	--	--	--
Formamide	--	--	--	--	3.014	--	--
Propionamide	--	--	--	--	--	4.803	--
Ammonium formate	--	--	--	--	--	--	4.154
Total	100.000	100.000	100.000	106.034	100.814	102.603	101.954
Per cent protein	11.60	5.87	11.60	11.60	11.60	11.60	11.60

lambs were placed in the metabolism cages. This procedure was followed until collections had been made from all the lambs.

The lambs on this trial were fed in individual feeding stalls. The animals were placed in the stalls in the morning and evening for periods of from two to three hours after which they were turned out into the pens. The lambs were fed ad libitum from individual self feeders during the two daily feeding periods. The lambs were weighed at the beginning of the experiment and every 14 days thereafter until the experiment was concluded.

Results and discussion

At the beginning of the experiment it was intended that formamide be included as one of the compounds tested. However, it became apparent almost immediately that the lambs receiving this compound would not survive if they continued to eat the ration containing it. Reference to Table 19 will indicate that during the first 14 days of the trial the lambs consumed only small amounts of the formamide ration and lost considerable weight during this period. In addition all the animals receiving formamide developed a peculiar paralysis of the rear legs severely limiting their ability to move about. Lamb 13 was removed from the feeding pen

Table 19. Summary of results with feeding formamide to lambs

Lamb no.	Original weight 2/4/53	Weight 2/18/53	Av. daily rate of gain	Av. daily feed consumption
13	70	60	-.71	.88
23	61	53	-.57	.82
33	64	53	-.79	.66
43	70	Removed from trial 2/13/53		
53	74	62	-.86	.57

after developing paralysis and appeared to be recovering, but after being placed back on the formamide ration suffered a rapid relapse and died. In view of probable fatal results occurring with continued feeding of formamide it seemed advisable to discontinue feeding this compound.

Table 20 contains a summary of the feed consumption by the lambs during the trial. It will be noted that two lambs from the urea lot died during the course of the experiment. Lamb 31 died quite suddenly about four weeks after the trial began. The lamb was not autopsied because it was believed that the time lapse between death and autopsy was too great to allow for any precise determination of the cause of death. Lamb 11 died two days before the end of the experiment. This lamb's feed consumption began to

Table 20. Average daily feed consumption (expressed in pounds)

Ration	Lamb no.	Total feed consumed	Total days on feed	Average daily feed consumption	
Urea	11	226.56 Died 5/11/53	96	2.36	
	21	293.02	98	2.99	
	31	Died 3/2/53	--	--	
	41	299.88	98	3.06	
	51	188.16	98	1.92	
	Av. for ration				2.58
Ammonium propionate	12	232.26	98	2.37	
	22	165.62	98	1.69	
	32	158.76	98	1.62	
	42	190.12	98	1.94	
	52	197.96	98	2.02	
	Av. for ration				1.93
Propionamide	14	257.74	98	2.63	
	24	322.42	98	3.29	
	34	206.78	98	2.11	
	44	306.74	98	3.13	
	54	324.38	98	3.31	
	Av. for ration				2.89
Ammonium formate	15	283.22	98	2.89	
	25	222.46	98	2.27	
	35	338.10	98	3.45	
	45	233.24	98	2.38	
	55	173.46	98	1.77	
	Av. for ration				2.55
Low nitrogen	16	252.84	98	2.58	
	26	251.86	98	2.57	
	36	207.76	98	2.12	
	46	342.02	98	3.49	
	56	357.70	98	3.65	
	Av. for ration				2.88
Conventional	17	310.66	98	3.17	
	27	329.28	98	3.36	
	37	267.54	98	2.73	
	47	337.12	98	3.44	
	57	301.84	98	3.08	
	Av. for ration				3.16

decline while in the metabolism cage and declined steadily until his death. The autopsy report upon this lamb indicated that the animal probably died from a toxemia resulting from a stasis of the intestinal tract.

It may be seen from Table 20 that all lambs ate less of their respective rations during the first two weeks of the trial than they did subsequently. This tendency was not so pronounced with the lambs receiving the conventional ration. The lambs on the conventional ration consumed the most feed with an average feed consumption of 3.16 pounds per day followed by the propionamide lambs, 2.89 pounds; low nitrogen, 2.88 pounds; urea, 2.59 pounds; ammonium formate, 2.55 pounds; and ammonium propionate, 1.93 pounds. This table includes the feed consumed by the lambs while they were in the metabolism cages. A separate summary of the feed consumption during the time the lambs were in the metabolism cages appears in Table 21. From a comparison of the two tables it may be seen that with the exception of the propionamide lambs the animals consumed only slightly less feed while in the metabolism cages than when in the feeding pens.

An enumeration of average daily gains in body weight appears in Table 22. The blank spaces in this table indicate that the lambs were in the metabolism cages on the regular day for weighing. The lambs receiving the

Table 21. Feed consumption while lambs in metabolism cages (expressed in pounds)

Ration	Lamb no.	Total feed consumed	Total days on feed	Average daily feed consumption	
Urea	11	22.29	10	2.23	
	21	28.76	10	2.88	
	31	Died early in trial	--	--	
	41	31.30	10	3.13	
	51	20.48	10	2.05	
	Av. for ration				2.57
Ammonium propionate	12	29.03	10	2.90	
	22	15.29	10	1.53	
	32	11.84	10	1.18	
	42	16.35	10	1.64	
	52	11.97	10	1.20	
	Av. for ration				1.69
Propionamide	14	25.31	10	2.53	
	24	25.62	10	2.56	
	34	14.78	10	1.48	
	44	24.69	10	2.47	
	54	18.92	10	1.89	
	Av. for ration				2.19
Ammonium formate	15	37.33	10	3.73	
	25	23.19	10	2.32	
	35	23.68	10	2.37	
	45	21.63	10	2.16	
	55	19.11	10	1.91	
	Av. for ration				2.49
Low nitrogen	16	35.64	10	3.56	
	26	24.67	10	2.47	
	36	19.33	10	1.93	
	46	26.60	10	2.66	
	56	30.81	10	3.08	
	Av. for ration				2.74
Conventional	17	31.41	10	3.14	
	27	28.04	10	2.80	
	37	29.46	10	2.95	
	47	32.52	10	3.25	
	57	24.44	10	2.44	
	Av. for ration				2.92

Table 22. Average daily gain in body weight

Ration	Lamb no.	Original weight	Final weight	Total pounds gained	Total days on feed	Average daily gain	
Urea	11	73	81	8	96	.08	
	21	56	86	30	98	.31	
	31	66 Died 3/2/53		--	--	--	
	41	67	98	31	98	.32	
	51	75	71	-4	98	-.05	
	Av. for ration						.17
Ammonium propionate	12	72	84	22	98	.22	
	22	61	69	8	98	.08	
	32	66	77	11	98	.11	
	42	67	83	16	98	.16	
	52	77	91	14	98	.14	
	Av. for ration						.14
Propionamide	14	70	99	29	98	.30	
	24	62	101	39	98	.40	
	34	65	73	8	98	.08	
	44	68	98	30	98	.31	
	54	75	103	28	98	.29	
	Av. for ration						.27
Ammonium formate	15	73	102	29	98	.30	
	25	58	75	17	98	.17	
	35	66	93	27	98	.28	
	45	68	86	18	98	.18	
	55	72	81	9	98	.09	
	Av. for ration						.20
Low nitrogen	16	68	86	18	98	.18	
	26	60	76	16	98	.16	
	36	66	77	11	98	.11	
	46	69	92	23	98	.23	
	56	74	97	23	98	.23	
	Av. for ration						.19
Conventional	17	70	103	33	98	.34	
	27	61	105	44	98	.45	
	37	62	88	26	98	.27	
	47	69	101	32	98	.33	
	57	76	114	38	98	.39	
	Av. for ration						.35

conventional ration gained .35 pounds per day; the propionamide lambs .27 pounds; the ammonium formate lambs .20 pounds; the lambs receiving the low nitrogen ration .19 pounds; the lambs receiving urea .17 pounds and the lambs on the ammonium propionate ration .14 pounds per day.

In feed efficiency the conventional ration ranked first (see Table 23) followed by propionamide, ammonium formate, ammonium propionate, urea and the low nitrogen ration with values of feed per pound gain of 9.13, 12.96, 13.52, 14.34, 15.50 and 15.82, respectively.

Nitrogen balance data were obtained on all lambs for the seven-day collection period. Total feces collected was saved and dried whereas only ten per cent aliquots of urine were saved. Samples of the urine and feces were subsequently analyzed for nitrogen content. Samples of feed from batches fed to the lambs during the collection period were also saved and analyzed for nitrogen as were the orts or left-over portions of feed remaining in the feed boxes at the end of the collection period. Samples of the feed and feces were oven dried before analysis.

Tables 24 to 29 include summaries of the nitrogen balance data obtained in this experiment. Four calculations were made (1) nitrogen balance in grams, (2) per cent digestibility of nitrogen, (3) per cent nitrogen retained of that absorbed, and (4) per cent nitrogen retained of

Table 23. Feed efficiency of lambs fed non-protein nitrogen compounds (expressed in pounds)

Ration	Lamb no.	Total feed consumed	Total weight gained	Lbs. feed per lb. gain	
Urea	11	226.56	8.00	28.32	
	21	293.02	30.00	9.77	
	31	--	--	--	
	41	299.88	31.00	9.67	
	51	188.16	-4.00	--	
	Av. for ration				15.50
Ammonium propionate	12	232.26	22.00	10.56	
	22	165.62	8.00	20.70	
	32	158.76	11.00	14.43	
	42	190.12	16.00	11.88	
	52	197.96	14.00	14.14	
	Av. for ration				14.34
Propionamide	14	257.74	29.00	8.89	
	24	322.42	39.00	8.27	
	34	206.78	8.00	25.85	
	44	306.74	30.00	10.22	
	54	324.38	28.00	11.58	
	Av. for ration				12.96
Ammonium formate	15	283.22	29.00	9.77	
	25	222.46	17.00	13.09	
	35	338.10	27.00	12.52	
	45	233.24	18.00	12.96	
	55	173.46	9.00	19.27	
	Av. for ration				13.52
Low nitrogen	16	252.84	18.00	14.05	
	26	251.86	16.00	15.74	
	36	207.76	11.00	18.89	
	46	342.02	23.00	14.87	
	56	357.70	23.00	15.55	
	Av. for ration				15.82
Conventional	17	310.66	33.00	9.41	
	27	329.28	44.00	7.48	
	37	267.54	26.00	10.29	
	47	337.12	32.00	10.54	
	57	301.84	38.00	7.94	
	Av. for ration				9.13

Table 24. Nitrogen balance data for urea ration fed to lambs

Lamb no.	21	51	41	11
Dry feed consumed, gms.	6731.42	4889.81	7538.83	5529.38
Nitrogen in feed, gms.	163.57	116.38	179.42	129.94
Nitrogen in orts, gms.	2.47	--	16.11	20.91
Dry feces, gms.	2114.84	1370.04	2160.06	1308.94
Nitrogen in feces, gms.	55.62	36.03	61.13	33.25
Urine, ml.	5050	5383	7045	3423
Nitrogen in urine, gms.	59.15	52.62	78.85	63.62
Nitrogen balance in gms.	46.33	27.73	23.33	12.16
% digest of nitrogen	65.47	69.04	62.57	69.50
% N retained of absorbed	43.92	34.51	22.83	16.05
% N retained of consumed	28.76	23.83	14.29	11.15

Table 25. Nitrogen balance data for ammonium propionate ration fed to lambs

Lamb no.	22	52	42	32	12
Dry feed consumed, gms.	3352.10	2712.53	3742.20	3229.63	6722.35
Nitrogen in feed, gms.	74.75	59.68	82.33	71.05	148.56
Nitrogen in orts, gms.	--	--	16.19	26.71	--
Dry feces, gms.	800.66	589.05	969.90	653.65	2004.46
Nitrogen in feces, gms.	23.14	15.08	28.81	19.15	52.52
Urine, ml.	8406	9402	3322	3003	5562
Nitrogen in urine, gms.	44.24	44.21	39.91	32.80	57.80
Nitrogen balance in gms.	7.37	0.29	-2.58	-7.61	38.24
% digest of nitrogen	69.04	74.73	56.44	56.81	64.65
% N retained of absorbed	14.28	0.87	--	--	39.82
% N retained of consumed	9.86	0.65	--	--	25.74

Table 26. Nitrogen balance data for propionamide ration fed to lambs

Lamb no.	24	54	44	34	14
Dry feed consumed, gms.	5697.22	4381.78	5007.74	3206.95	5760.72
Nitrogen in feed, gms.	112.80	81.50	93.14	59.65	107.15
Nitrogen in orts, gms.	1.71	5.35	16.94	21.26	16.56
Dry feces, gms.	1690.51	1285.54	1420.00	616.07	1392.12
Nitrogen in feces, gms.	42.43	34.32	39.76	18.85	37.17
Urine, ml.	6003	4405	3861	3009	4758
Nitrogen in urine, gms.	56.19	63.45	55.63	45.60	55.62
Nitrogen balance in gms.	12.47	-21.62	-19.19	-26.06	-2.20
% digest of nitrogen	61.81	54.93	47.82	50.90	58.97
% N retained of absorbed	18.16	--	--	--	--
% N retained of consumed	11.23	--	--	--	--

Table 27. Nitrogen balance data for ammonium formate ration fed to lambs

Lamb no.	25	55	45	35	15
Dry feed consumed, gms.	5030.42	4218.48	4776.41	5298.05	7865.42
Nitrogen in feed, gms.	116.71	91.54	103.65	114.97	170.68
Nitrogen in orts, gms.	2.00	--	--	19.16	15.74
Dry feces, gms.	1474.90	1013.76	1362.81	1530.45	2125.00
Nitrogen in feces, gms.	37.46	25.55	37.89	42.70	59.71
Urine, ml.	5203	8153	8575	5011	6729
Nitrogen in urine, gms.	50.70	55.50	51.41	66.65	74.00
Nitrogen balance in gms.	26.55	10.49	14.35	-13.54	21.23
% digest of nitrogen	67.34	72.09	63.44	55.43	61.46
% N retained of absorbed	34.37	15.90	21.82	--	22.29
% N retained of consumed	23.15	11.46	13.84	--	13.70

Table 28. Nitrogen balance data for low nitrogen ration fed to lambs

Lamb no.	26	56	46	36	16
Dry feed consumed, gms.	5769.79	7720.27	6550.00	5066.71	8618.40
Nitrogen in feed, gms.	81.90	102.68	87.12	67.39	114.62
Nitrogen in orts, gms.	3.65	2.36	11.72	5.47	12.83
Dry feces, gms.	1794.46	2797.94	1859.95	1398.11	3229.14
Nitrogen in feces, gms.	47.37	61.83	44.27	35.23	62.00
Urine, ml.	3475	5540	5092	3847	4414
Nitrogen in urine, gms.	11.30	17.72	14.78	11.15	15.00
Nitrogen balance in gms.	19.58	20.77	16.35	15.54	24.79
% digest of nitrogen	39.46	38.27	41.29	43.10	39.09
% N retained of absorbed	63.41	53.96	52.52	58.41	62.30
% N retained of consumed	25.02	20.70	21.68	25.10	24.35

Table 29. Nitrogen balance data for conventional ration fed to lambs

Lamb no.	27	57	47	37	17
Dry feed consumed, gms.	6794.93	6173.50	8246.45	7493.47	7715.74
Nitrogen in feed, gms.	157.64	141.37	188.84	199.33	205.24
Nitrogen in orts, gms.	2.49	12.98	9.50	21.18	19.12
Dry feces, gms.	2111.90	1730.43	2782.00	2089.89	2056.19
Nitrogen in feces, gms.	56.39	49.84	75.67	62.91	59.84
Urine, ml.	4024	5387	4433	5229	5972
Nitrogen in urine, gms.	60.64	56.01	70.48	76.32	105.27
Nitrogen balance in gms.	38.12	23.54	33.19	38.92	20.91
% digest of nitrogen	63.65	61.48	57.81	64.69	67.83
% N retained of absorbed	38.60	29.59	32.02	33.79	16.57
% N retained of consumed	24.57	18.19	13.04	21.85	11.24

that consumed. Table 30 contains a summary of all the nitrogen balance data.

The nitrogen balance data indicated that all but seven lambs maintained a positive nitrogen balance during the seven-day collection periods. Of these seven animals four were receiving propionamide, two were receiving ammonium propionate and one was receiving ammonium formate. The average nitrogen balance for the five lambs receiving each ration was positive for every ration except the propionamide ration. A factor contributing to the negative nitrogen balance of the lambs receiving propionamide was that their feed consumption dropped considerably when they were placed in the metabolism cages.

Table 30. Summary of nitrogen balance data for lambs fed non-protein nitrogen compounds

	Av. 7-day N balance in grams	% nitrogen digested	% nitrogen retained of absorbed	% nitrogen retained of consumed
Urea	27.39	66.64	29.33	19.51
Ammonium propionate	7.16	64.33	14.07	9.10
Propionamide	-11.32	54.89	--	--
Ammonium formate	11.82	63.95	16.53	10.54
Low nitrogen	19.41	40.24	58.12	23.37
Conventional	30.94	63.09	30.11	17.78

The figures in Table 30 indicate that there was not a great difference in the digestibility of the nitrogen from the four non-protein nitrogen compounds although the digestibility of the nitrogen from the propionamide was somewhat lower than the other three. The lambs receiving the low nitrogen ration showed the lowest nitrogen digestibility of all. This may have been in part due to an interruption of the absorptive processes of the intestine since most of the lambs on this ration suffered from diarrhea.

Of the animals receiving the non-protein nitrogen compounds, the lambs on the urea ration retained the highest percentage of nitrogen of that absorbed and of that consumed. In these respects the urea lambs compared quite favorably with the lambs receiving the conventional ration--retaining 29.33 per cent of the nitrogen absorbed and 19.51 per cent of the nitrogen consumed as compared with 30.11 per cent and 17.78 per cent for the lambs on the conventional ration. The nitrogen from ammonium formate was retained in the next highest percentage among the non-protein nitrogen compounds tested followed by ammonium propionate. Since the lambs fed propionamide were in negative nitrogen balance a net loss of nitrogen was indicated for these lambs. The lambs fed the low nitrogen ration retained the highest percentage of the nitrogen absorbed, 58.12 per cent and of nitrogen consumed 23.37 per cent. This is

understandable since it is a common observation that animals on a low protein ration increase their efficiency of nitrogen utilization.

Obtaining the nitrogen balance data was the primary objective of this experiment and feeding results were considered secondary. This was true because serious interruptions of the feeding trial resulted when lambs were transferred to metabolism cages and back again to feeding pens. Therefore the results obtained on feed consumption, rate of gain and feed efficiency were not considered to reflect the true feeding value of the rations used.

On the basis of the nitrogen balance data, however, it was concluded that a higher percentage of urea nitrogen is retained than the nitrogen from the other non-protein nitrogen compounds tested. It should be remembered, however, that two of the five urea lambs died during the experiment from symptoms which suggested ammonia toxicity. The other non-protein nitrogen compounds did not compare as favorably with the conventional ration in nitrogen retention as did the urea ration. However, the amount of nitrogen retained by the lambs in this experiment was not directly correlated with rate of gain especially in the case of the propionamide ration where the lambs gained well in body weight but were in negative nitrogen balance during the time they were in the metabolism cages. This may indicate that further

nitrogen balance studies will be needed to explain these results.

Summary

Thirty-five western wether lambs were alloted into seven groups according to weight. Each group was fed a different ration. Five of these rations contained each of one of the following non-protein nitrogen compounds: urea, ammonium propionate, propionamide, ammonium formate and formamide in amounts sufficient to furnish 50 per cent of the nitrogen of the ration. One of the two remaining rations was a conventional lamb fattening ration and the other was similar to the rations containing the non-protein nitrogen compounds except that the non-protein nitrogen compound was deleted. During the trial each of the lambs was placed in a metabolism cage for a three-day preliminary and a seven-day collection period for determination of nitrogen balance.

The lambs receiving the conventional ration consumed the most feed, gained the most weight and required the least feed per pound of gain. In feed consumption the other lots ranked as follows: propionamide, low nitrogen, urea, ammonium formate and ammonium propionate. The average daily gains in body weight as recorded were: propionamide

lambs, .27 pounds; ammonium formate lambs, .20 pounds; low nitrogen lambs, .19 pounds; urea lambs, .17 pounds; and ammonium propionate lambs, .12 pounds.

The lambs receiving urea had the highest percentage of nitrogen retention of the lambs receiving any of the non-protein nitrogen compounds followed by the lambs receiving ammonium formate, ammonium propionate and propionamide. The variation in per cent nitrogen digested was not great among any of the non-protein nitrogen rations. Although the lambs which received propionamide were in negative nitrogen balance during the collection period, they actually made the next highest rate of gain over the entire experimental period. Less than one pound of the formamide ration was consumed daily by the lambs during the time which it was fed, and all lambs on this ration developed a paralysis of the hind legs requiring that they be removed from the experiment.

Adaptability of Sheep Rumen Microorganisms to Propionamide as Measured by the Rate of Ammonia Release *In Vitro*

In the three feeding trials which preceded this part of the study the lambs receiving propionamide as a partial source of dietary nitrogen gained an average of .33 pounds

daily in body weight. This amount was appreciably higher than the .19 pounds gained by the lambs receiving the low nitrogen negative control rations. These results indicated that the lambs receiving the propionamide were utilizing some of the nitrogen from that compound for growth and fattening. The results of the toxicity studies, however, had shown that among animals which had not previously received propionamide in the ration, essentially no ammonia nitrogen was released into the blood following oral administration of that compound. In view of these results it was thought that the rumen microorganisms of lambs receiving dietary propionamide adapted themselves to the utilization of propionamide nitrogen after a certain time. This phase of the study was instituted to determine whether such an adaptation was made by the microorganisms and if so, how long a period was necessary for maximum adaptation.

Materials and methods

A total of 13 lambs were used in this phase of the study. Six of the lambs were fed a conventional lamb fattening ration while the remaining seven lambs were fed a ration in which propionamide furnished approximately 50 per cent of the dietary nitrogen. These seven lambs received this ration for varying lengths of time as follows: 4, 11,

15, 20, 96, 106 and 110 days. Both the lambs fed the conventional ration and those receiving the propionamide ration were slaughtered and their entire rumen contents were removed. Rumen liquor was extracted through gauze and processed by the method as developed by Burroughs et al. (4) and modified by Cheng (7). This procedure included centrifuging the rumen liquor at 3000 revolutions per minute for the purpose of removing remaining feed particles and protozoa; and then concentrating the bacterial cells and at the same time removing nutrient material by centrifuging the cells at 5000 revolutions per minute for 20 minutes.

The cells were then dispersed in a basal mineral solution which was a modification of the solution developed by Burroughs et al. (5) and which resembles sheep saliva in composition. Approximately one per cent of pure wood cellulose was added and the entire suspension apportioned into fermentation tubes containing 20 milliliters each. To each fermentation tube was added enough urea or propionamide to furnish 10 milligrams of nitrogen. The fermentation tubes were incubated in a water bath at 40° C. Carbon dioxide was bubbled through the fermentation tubes during the fermentation period. At intervals of 2, 4, 8, 12 and 24 hours fermentation tubes were removed and centrifuged for five minutes at 5000 revolutions per minute and an aliquot of the supernatant liquid removed and analyzed for ammonia nitrogen by

the method of Van Slyke and Cullen (38). By using control tubes to which no non-protein nitrogen compounds were added it was possible to determine by difference the percentage of nitrogen released of the total amount of nitrogen added as urea or propionamide. By this procedure it was possible to determine the rate at which the ammonia nitrogen was released within the 24-hour period during which samples from the fermentation tubes were taken. It also offered a basis of comparison between the rate of release of nitrogen by rumen bacteria from lambs not previously fed propionamide and lambs which had received it in the diet previously. Nine lambs were used in this part of the experiment, four which had been receiving a conventional ration previous to slaughter and five lambs which had received propionamide in the ration for 4, 11, 15, 20 and 110 days, respectively.

The percentage of cellulose digested by the rumen bacteria from nine of the 13 lambs used in this experiment was also measured. The rumen bacteria from three lambs not previously fed propionamide and from six lambs fed propionamide for 4, 11, 20, 96, 106 and 110 days, respectively, were employed in determining the cellulose digestion. The fermentation period was 24 hours.

Results and discussion

Figure 10 and Table 31 summarize the data on the rate of ammonia nitrogen released from propionamide by rumen microorganisms from four lambs previously fed a conventional lamb fattening ration and five lambs fed propionamide for 4, 11, 15, 20 and 110 days previous to slaughter and removal of rumen contents. It can be seen that with the exception of the bacteria from the lamb receiving propionamide for 15 days the percentage ammonia nitrogen released was greater by the bacteria from the lambs which had received propionamide than from the control lambs.

The lower two curves in Figure 11 represent the average percentages of ammonia nitrogen released by the rumen bacteria from the four control lambs and from the five lambs fed propionamide whose individual curves appeared in Figure 10. The two curves at the top of Figure 11 represent the average percentage of ammonia nitrogen released from urea by the same nine lambs. The individual values for NH_3-N release from urea by the rumen bacteria appear in Table 32. Analysis of variance of the percentage ammonia nitrogen release from propionamide revealed a highly significant increase ($P < .01$) in percentage of ammonia nitrogen released by the rumen bacteria from the lambs previously fed propionamide. Analysis of this data also indicated that the

Figure 10. *In Vitro* release of NH_3-N from propionamide by rumen bacteria both from lambs previously fed and not previously fed propionamide

Legend:

_____ average release by rumen bacteria from four lambs not previously fed propionamide

---------- release by rumen bacteria from one lamb fed propionamide for four days

__ __ __ release by rumen bacteria from one lamb fed propionamide for 11 days

___ - ___ release by rumen bacteria from one lamb fed propionamide for 15 days

___---___ release by rumen bacteria from one lamb fed propionamide for 20 days

___--___ release by rumen bacteria from one lamb fed propionamide for 110 days

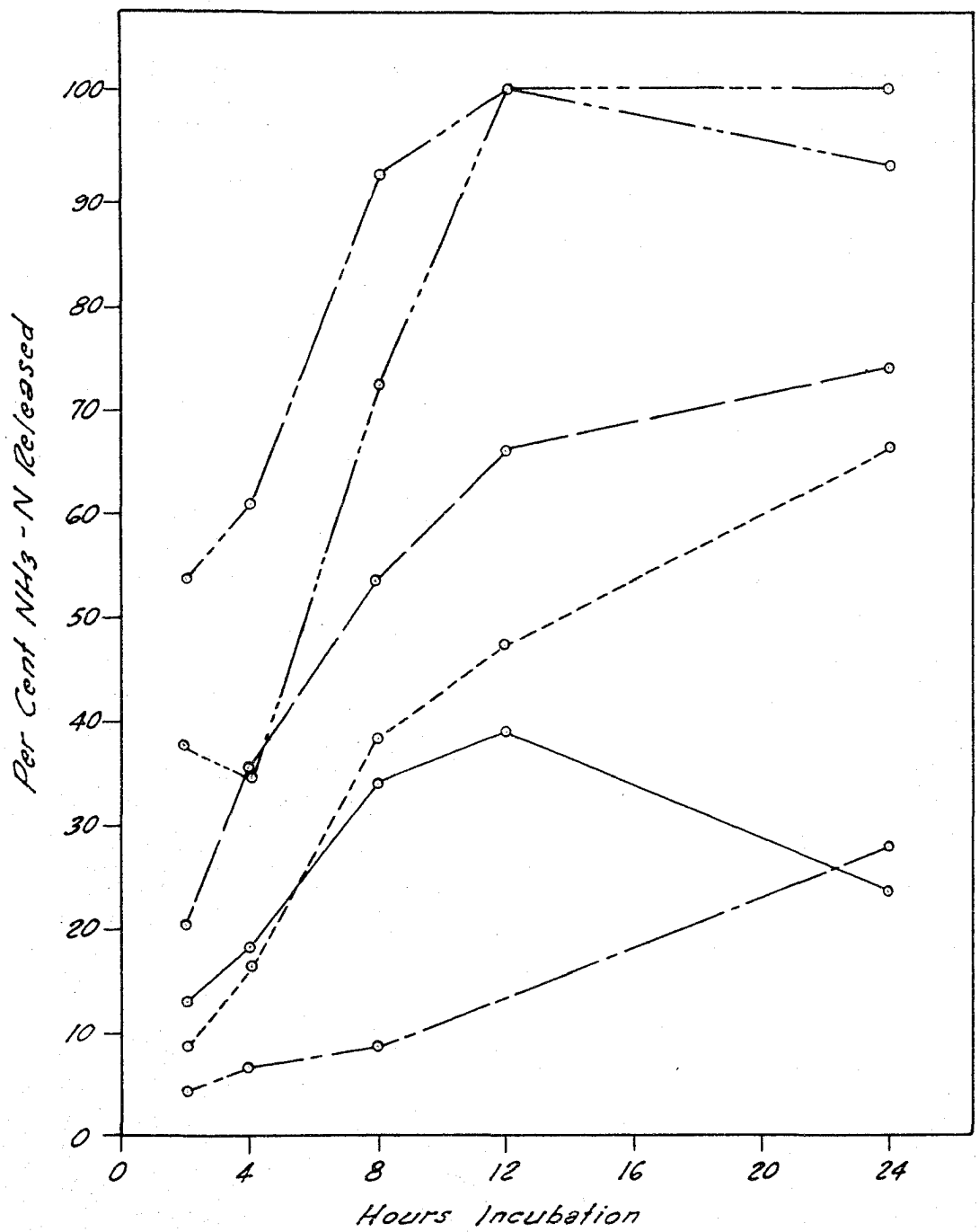

Per Cent NH3 - N Released
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Hours Incubation

Figure 10.

Table 31. *In Vitro* release of NH_3-N from propionamide by rumen bacteria both from lambs previously fed and not previously fed propionamide

No. of lambs	No. of days fed propionamide	Per cent NH_3-N released from propionamide at indicated intervals				
		2 hours	4 hours	8 hours	12 hours	24 hours
1	0	18.50	25.68	41.50	54.10	18.40
1	0	14.10	19.53	21.29	25.25	10.57
1	0	7.76	8.00	17.50	19.50	7.85
1	0	11.30	19.72	53.32	55.85	60.00
4 (av. of above 4 animals)		12.92	18.23	33.40	38.68	24.22
1	4	20.47	35.60	54.13	66.19	73.82
1	11	8.66	16.13	38.45	47.82	66.20
1	15	4.20	4.20	6.46	8.63	28.17
1	20	53.80	60.92	92.50	100.00	93.40
1	110	37.30	35.20	72.40	100.00	100.00
5 (av. of above 5 animals)		24.89	30.41	52.79	64.53	72.32

Figure 11. Average *In Vitro* release of NH_3-N from propionamide and urea by rumen bacteria both from lambs previously fed and not previously fed propionamide

Legend:

———— average release from propionamide by rumen bacteria from four lambs not previously fed propionamide

— — — average release from propionamide by rumen bacteria from five lambs previously fed prionamide for 4, 11, 15, 20 and 110 days, respectively

— - — average release from urea by rumen bacteria from four lambs not previously fed propionamide

---------- average release from urea by rumen bacteria from five lambs previously fed propionamide for 4, 11, 15, 20 and 110 days, respectively

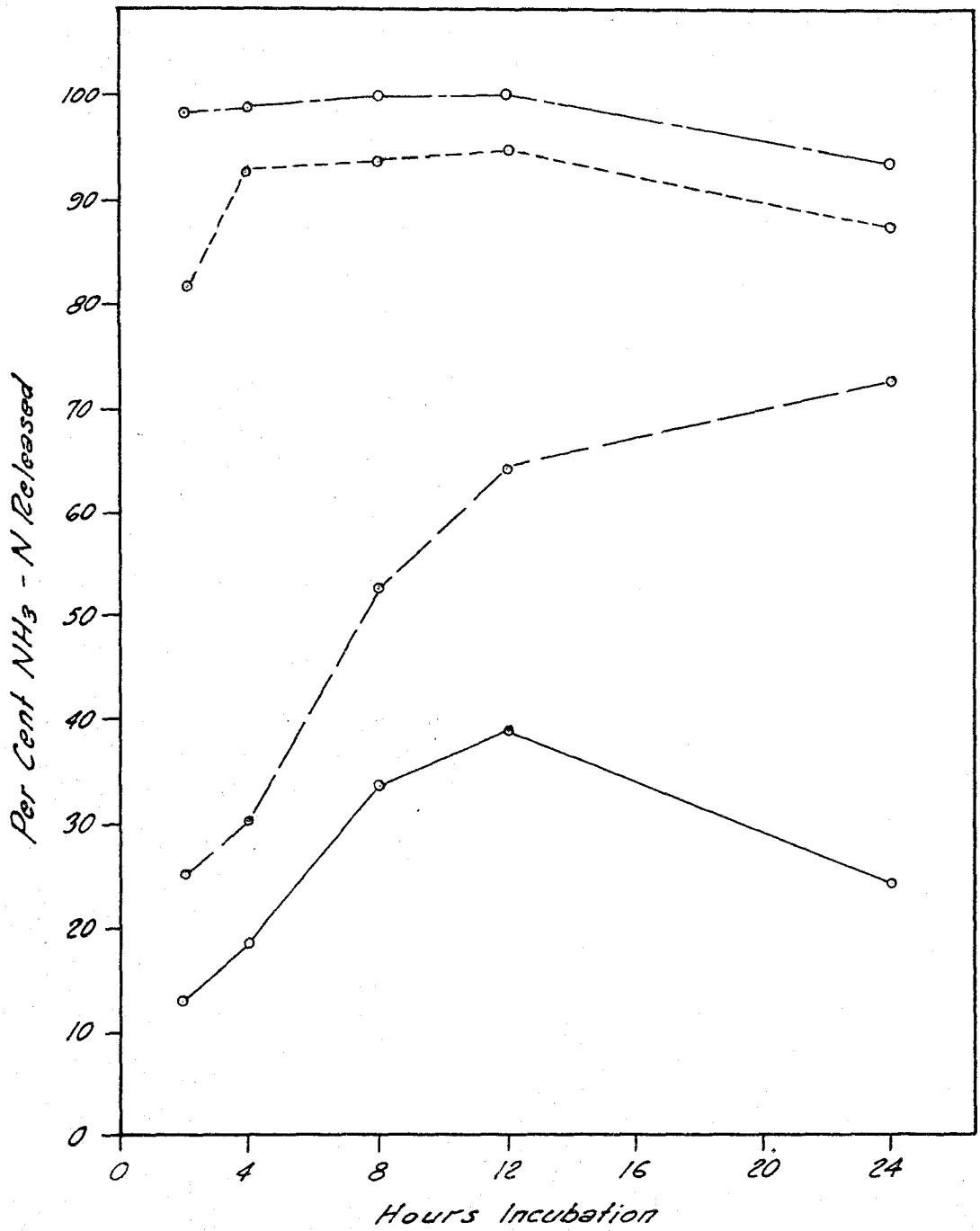

Per Cent NH_3 - N Released
100
90
80
70
60
50
40
30
20
10
0
0
4
8
12
16
20
24
Hours Incubation

Figure 11.

Table 32. Average *In Vitro* release of NH_3-N from urea by rumen bacteria both from lambs previously fed and not previously fed propionamide

No. of lambs	No. of days fed propionamide	Per cent NH_3-N released from urea at indicated intervals				
		2 hours	4 hours	8 hours	12 hours	24 hours
1	0	100.00	100.00	100.00	100.00	100.00
1	0	100.00	100.00	100.00	100.00	94.35
1	0	96.70	96.70	100.00	100.00	83.85
1	0	99.12	100.00	100.00	100.00	95.60
4 (av. of above 4 animals)		98.96	99.18	100.00	100.00	93.45
1	4	82.90	100.00	100.00	97.80	85.20
1	11	89.35	91.38	94.86	100.00	88.00
1	15	69.40	71.85	72.75	75.39	72.83
1	20	100.00	100.00	100.00	100.00	88.70
1	90	67.20	100.00	100.00	100.00	100.00
5 (av. of above 5 animals)		81.77	92.65	93.52	94.64	86.95

differences between percentage of ammonia nitrogen released at the five sampling intervals were significant ($P < .05$) indicating a continuing increase of ammonia nitrogen release with time. The two curves representing the ammonia nitrogen release from urea showed that nearly all the nitrogen was released from the urea within the first two hours of incubation and that only a small but consistent difference existed between the amount of ammonia nitrogen released by the bacteria from the control and propionamide-fed lambs. This rapid rate of release of nitrogen from urea in this experiment is in agreement with the findings of Pearson and Smith (31).

The results of the adaptation phase of this experiment as summarized in Figures 10 and 11 indicated that the rumen microflora adjusted themselves to the utilization of propionamide nitrogen after having received this compound for about three weeks. The conclusion that adaptation of the microflora took place is in agreement with the results of previous feeding experiments in which lambs receiving this compound had always made satisfactory weight gains. The rate of release of ammonia from urea was essentially the same by microorganisms from lambs whether they had received propionamide previously or not. The rate of release of ammonia from urea was more rapid than from propionamide. When the lambs became fully adapted to the propionamide

(after receiving it for at least 20 days) their rumen microorganisms were able to release the ammonia nitrogen from this compound at approximately one-half the rate at which they released it from urea.

The percentage cellulose digestion by rumen bacteria from three control lambs not fed propionamide is recorded in Table 33. The figures in this table indicate that a considerably higher percentage of cellulose was digested by the bacteria which received urea as a nitrogen source than by the bacteria either receiving no added nitrogen or nitrogen in the form of propionamide. The bacteria receiving the propionamide digested approximately five per cent more cellulose than the bacteria receiving no added nitrogen.

Comparison of the data in Tables 33 and 34 shows that rumen bacteria from lambs which were fed propionamide for varying lengths of time digested a slightly higher percentage of cellulose when propionamide was added to the fermentation media than did lambs which had not previously been fed propionamide. The difference in cellulose digestion between the bacteria receiving urea as a nitrogen source and those receiving propionamide was considerably less (64.76 per cent and 63.08 per cent) for the lambs which had received propionamide in the ration than for the lambs which had not received propionamide in the ration (75.50 per cent

Table 33. Percentage cellulose digestion by sheep rumen microorganisms *In Vitro* using urea and propionamide as nitrogen sources

A. Animals not previously fed propionamide

No. of lambs sampled	Nitrogen source		
	No nitrogen added (control)	Urea	Propionamide
1	50.22	81.21	62.54
1	60.20	70.93	63.06
1	54.75	74.35	54.28
3 (av. of samples above)	55.06	75.50	59.96

Table 34. Percentage cellulose digestion by sheep rumen microorganisms *In Vitro* using urea and propionamide as nitrogen sources

B. Animals previously fed propionamide

No. of lambs sampled	No. of days fed propionamide	Nitrogen source		
		No nitrogen added (control)	Urea	Propionamide
1	4	34.41	50.83	45.76
1	11	49.20	38.84	48.51
1	20	55.85	72.25	60.00
1	96	56.72	77.63	71.37
1	106	75.23	75.50	78.24
1	110	67.11	73.52	74.26
6 (av. of above animals)		56.42	64.76	63.02

and 59.96 per cent).

It is believed that greater differences in cellulose digestion could have been recorded had it been possible to remove a greater percentage of the nitrogen from the bacterial cell suspension. An attempt was made to decrease this residual nitrogen by washing the cells twice or even once with distilled water. When this procedure was followed essentially no cellulose digestion was recorded. Therefore the less rigorous method of merely centrifuging the bacterial cells and removing the supernatant liquid was followed. Using this method, however, resulted in allowing from .5 to 7 milligrams of nitrogen to remain per 20 milliliter fermentation flask.

The data on cellulose digestion was certainly not conclusive, but it may be considered suggestive that when propionamide is added to the fermentation media cellulose digestion by rumen microorganisms is improved among lambs which have previously been fed this compound as compared to those lambs which have not. The reason for the increased cellulose digestion is presumably due to the greater release of nitrogen by the rumen microflora as they become adapted to its presence.

Summary

Six lambs which had received a conventional lamb fattening ration and seven lambs which had received a ration containing propionamide in an amount such as to furnish approximately 50 per cent of the protein equivalent for 4, 11, 15, 20, 96, 106 and 110 days were slaughtered and their entire rumen contents removed. The rumen bacteria from four of the lambs on the conventional ration and from five of the lambs on the propionamide ration were incubated with given amounts of urea or propionamide. Samples of the supernatant liquid from the fermentation flasks were removed at intervals and analyzed for ammonia nitrogen. It was found that ammonia nitrogen was released at a significantly greater rate from propionamide by bacteria from lambs that had previously received this compound in the ration. The rate of ammonia nitrogen release from propionamide was approximately one-half as fast as from urea.

It was found that rumen bacteria receiving nitrogen in the form of urea digested more cellulose than those receiving nitrogen from propionamide. This difference in cellulose digestion was much greater for the bacteria from lambs which had not previously received propionamide than for those which had received it in the ration. Bacteria from the propionamide-fed lambs digested a slightly greater

percentage of cellulose than did bacteria from lambs fed a conventional ration when propionamide was used as a nitrogen source.

GENERAL DISCUSSION

The ideal non-protein nitrogen compound would have several characteristics. First, it should be non-toxic. Second, every part of the molecule should be utilizable by the rumen microorganisms or by the animal. Third, it should be at least relatively palatable. Fourth, it should be cheap enough in price to compete with other nitrogen supplements.

Presumably, toxicity might be of two types, acute and chronic. The investigations reported in this thesis were concerned primarily with acute toxicity and only incidental observations related to chronic toxicity were made. It is felt, however, that systematic studies of the long-time effects of feeding non-protein nitrogen compounds upon such vital organs as the heart, liver and kidneys would be worthwhile.

Many cases of so-called urea toxicity have been reported from the field. Improper mixing of urea in the feed is usually considered responsible for animals consuming such amounts of urea as to cause acute toxicity. Although it is considered entirely possible that urea toxicity might be caused from overeating this compound in the feed, it is believed that this would happen infrequently. Urea is very bitter in taste and it was found in this study that animals

consumed daily in the feed for several months without ill effect amounts of urea sufficient to cause toxicity if administered as a drench. It is believed that the diagnosis of urea toxicity may sometimes have been in error. It is hoped that a simple and rapid test for high levels of ammonia in the blood may someday be feasible for use in diagnosing suspected cases.

The alleviation of symptoms of acute ammonia toxicity offers a field of investigation which was explored only partially in this thesis problem. It was found that by administering glacial acetic acid orally on an equivalent urea ammonia molar basis, symptoms of acute toxicity could be prevented or arrested even when the symptoms had proceeded to the point of collapse of the animal. It is believed that oral administration of acetic acid lowers the pH of the rumen and thereby lessens the rate at which ammonia is released from the non-protein nitrogen compounds. Clarification of the mechanism whereby acetic acid effects a reduction in the rate of ammonia release might furnish clues which would make it possible to use some of the more toxic non-protein nitrogen compounds in animal rations with safety. It might be that with the use of a urease inhibitor, for example, the rate of ammonia release from urea could be considerably reduced. It is also possible that the more toxic non-protein nitrogen compounds may be used with

greater safety with natural feeds of relatively low pH such as silages or with other feeds reduced in pH by acid treatment.

The rate of release of ammonia nitrogen from the non-protein nitrogen compounds by rumen microorganisms is critical in the evaluation of the efficiency of these compounds. If the rate of release is too rapid the compound has a relatively high toxicity since the nitrogen cannot be utilized as rapidly as it is released. If the rate of release is too slow the compound proceeds through the digestive tract essentially unchanged without utilization of the nitrogen. Ideally, the ammonia nitrogen should be released at a rate which provides for maximum utilization by the rumen bacteria being neither too rapid so as to be wasteful and toxic nor too slow so as to be insufficient to meet the requirements of the bacteria.

Measurement of blood ammonia and urea was used as a method for determining the rate at which ammonia nitrogen was released. The rate of ammonia release was also measured by determining the amount of ammonia nitrogen released from propionamide and urea by rumen microorganisms <u>in vitro</u> at various intervals throughout a fermentation period. The first method furnished information as to the relative toxicity of all the compounds tested, whereas the second method furnished information as to the relative availability of the

nitrogen from propionamide and urea. The in vitro studies with the two compounds were found to run parallel with the findings in the toxicity studies. The in vitro fermentation studies indicated a slow rate of release of ammonia from propionamide supporting the results of the blood analysis which showed only very small increases in blood ammonia after oral administration to the animal. The same agreement in results was recorded for urea in which case rapid release of ammonia in vitro was associated with rapid rises in blood ammonia in the live animal when this compound was used.

In order to evaluate the general efficiency of the non-protein nitrogen compounds, feeding trials, a nitrogen balance study and determination of cellulose digestion in vitro were employed as tehcniques. Results from these fields of investigation were not entirely consistent. Propionamide which supported slightly superior weight gains as compared to urea in lamb feeding trials did not allow lambs to maintain a positive nitrogen balance as did the urea-fed lambs. It should be stated, however, that in the nitrogen balance trial the lambs receiving propionamide consumed considerably less feed than their average feed consumption before and after being confined to the metabolism cages. Cellulose digestion in vitro was greater when urea was used as the nitrogen source than when propionamide was

used, however since propionic acid is formed upon the hydrolysis of propionamide the lower cellulose digestion recorded when this compound was used may have been caused in part by inhibition of bacterial function due to reduced pH.

A method of determining the efficiency of non-protein nitrogen compounds not used in this study but one which might serve to further explain the extent of their utilization in the rumen is concerned with the measurement of the proportion of the non-protein nitrogen which is actually utilized by the rumen bacteria. After allowing rumen bacteria to ferment in the artificial rumen for a prescribed period using the non-protein nitrogen compounds as nitrogen sources, the fermentation tubes could be centrifuged and a protein precipitant applied which would free the supernatant liquid of bacterial protein. The supernatant liquid could then be analyzed for ammonia nitrogen and with the use of proper control tubes the amount of nitrogen utilized by the rumen bacteria from the non-protein nitrogen compound could be determined. This procedure might afford another basis for evaluating non-protein nitrogen compounds.

All <u>in vivo</u> experiments were conducted with lambs, however it is realized that the greatest potential use for non-protein nitrogen compounds is in the feeding of cattle. This fact more than any other prompted the investigations

concerning the possible adaptation of rumen microorganisms to increasing utilization of the compounds after receiving them in the ration for some time. Since the fattening period for cattle is considerably longer than for lambs, an adaptation period if it were required would constitute a much smaller proportion of the fattening period for cattle. Lambs fed propionamide made satisfactory gains in all the feeding trials conducted, however, it of all the compounds fed was found to release very little ammonia into the blood when administered as a drench. These two findings suggested that adaptation to the utilization of propionamide nitrogen by the rumen microorganisms was taking place during the feeding period. This belief was confirmed by artificial rumen studies which showed a significantly greater release of ammonia nitrogen from propionamide by rumen bacteria from lambs having received propionamide in the ration as compared to those which had not. Nearly maximum adaptation was recorded in 20 days. This adaptation period was found to be in sharp contrast to the performance of urea from which ammonia was released extremely rapidly by rumen bacteria from lambs never having received this compound in the ration.

Another important consideration in the feeding of non-protein nitrogen compounds is the level at which to feed them. In the feeding trials reported in this thesis

satisfactory gains were recorded when the non-protein nitrogen compounds were fed at a level of 50 per cent of the protein equivalent. However, the highest gains recorded were made in the final individual feeding trial in which propionamide and urea were each fed at levels of 15 per cent and 30 per cent of the protein equivalent. It seemed significant that the lambs made rapid gains from the start of the experiment and did not appear to require an adaptation period. It would appear therefore that a feeding level of around 25 to 30 per cent of the protein equivalent of the ration may be more efficient than higher levels at least in the case of propionamide and urea. It is interesting to note that this level is about the amount that could be used to supplement many grain-hay cattle rations.

Of the non-protein nitrogen compounds investigated in this thesis problem propionamide appeared to be most promising in several respects. In the first place it was found to be non-toxic. In the second place lambs fed this compound gained as well as lambs fed any other compound and when fed at a level of 30 per cent of the protein equivalent gained essentially as well as those lambs fed a ration containing protein entirely from natural sources. Propionamide also has the advantage of having the energy containing propionic acid radical. Although found to produce acute toxicity, urea, ammonium formate and ammonium acetate supported

satisfactory weight gains among lambs and it is believed that if these compounds are thoroughly mixed in ruminant rations they could be fed with safety and efficiency up to levels of 50 per cent of the protein equivalent of the ration.

SUMMARY

Ten non-protein nitrogen compounds (urea, ammonium propionate, propionamide, ammonium formate, ammonium acetate, formamide, ammonium succinate, guanidine carbonate, biuret, glycine) and casein were administered orally to lambs for purposes of determining their relative toxicity. These compounds were administered in progressively larger doses and blood samples were taken at varying intervals following administration. Determinations of ammonia and urea were made for each blood sample. The blood ammonia values rose rapidly among all lambs except the control lambs and those receiving propionamide, ammonium succinate, guanidine carbonate, biuret, glycine and casein. Blood urea values rose much more gradually. In most cases toxicity was definitely associated with large increases in blood ammonia levels, with the critical level about 1 milligram per 100 milliliters. Administration of urea, ammonium propionate, ammonium formate and ammonium acetate at a level of 40 grams of urea (or its nitrogen equivalent of the other compounds) per 100 pounds body weight resulted in fatal toxicity. Fatal toxicity not associated with an increase in blood ammonia was observed after administration of guanidine carbonate at the 30 gram urea equivalent level. No increases in blood ammonia and only slight

increases in blood urea resulted from administration of propionamide even at levels of 80 grams urea equivalent when administered to lambs not having received this compound in the ration previously. However, when lambs had received propionamide in the ration for 49 days subsequent oral administration of 40 grams urea equivalent of propionamide resulted in slight increases in blood ammonia, and when 80 grams urea equivalent of propionamide were administered to these lambs moderate increases in blood ammonia were recorded. Only slight increases in blood ammonia and urea attended the administration of formamide at the 40 gram urea equivalent level or ammonium succinate at the 70 gram level. Practically no rise in blood ammonia or urea was recorded following the oral administration of guanidine carbonate, glycine, biuret or casein.

Research was also initiated to study selected non-protein nitrogen compounds as protein substitutes in lamb fattening rations. Two group feeding experiments, one individual feeding experiment, a nitrogen balance study and an <u>in vitro</u> adaptation study were conducted using the following compounds: urea, ammonium acetate, ammonium formate, ammonium propionate, propionamide and formamide. In the group feeding trials all non-protein nitrogen compounds were added in amounts necessary to furnish 50 per cent of the protein equivalent of the ration. Rates of gain with urea,

ammonium acetate, ammonium formate and propionamide compared favorably with the conventional protein ration. In an individual feeding trial conducted using propionamide at two levels (15 per cent and 30 per cent of the protein equivalent) and control rations of urea at the same levels, the rate of gain for the lambs fed propionamide at the 30 per cent level was essentially the same as for the conventional protein ration. In vitro studies to determine the rate at which sheep rumen microorganisms adapted themselves to the release of propionamide nitrogen after receiving it in the ration indicated full adaptation required about three weeks.

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